THE UNIVERSITY
of EDINBURGH

ASSESSMENT AND RISK PREDICTION IN PATIENTS WITH AORTIC STENOSIS

INSIGHTS FROM CARDIOVASCULAR MAGNETIC RESONANCE

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“A Parisian tailor, not yet old, having dined and left his house had walked hardly 40 paces when he suddenly fell to the ground and expired. His body was opened and no disease was found except the three semilunar cusps leading to the aorta were bony”

***Théophile Bonet**, 1679*

To Siska and Zoey,

CONTENTS

FIGURES INDEX

TABLES INDEX

ABSTRACT

BACKGROUND

Aortic stenosis affects not only the valve but also the myocardium. In response to the increased afterload, left ventricular hypertrophy initially occurs as a compensatory response to maintain wall stress and cardiac output but ultimately, decompensation and heart failure ensues. The transition from adaptation to decompensation is driven by myocyte death and myocardial fibrosis. The aims of the thesis are to investigate cardiovascular magnetic resonance assessment of disease severity and myocardial fibrosis, and explore its relationship with other biomarkers of disease activity and clinical outcome in patients with aortic stenosis.

METHODS AND RESULTS

The conventional assessment of aortic stenosis relies heavily on two-dimensional and Doppler echocardiography but there are inherent limitations in echocardiography that can affect the severity classification. I demonstrated that cardiovascular magnetic resonance offered a more accurate estimation of left ventricular volumes and mass, and excellent myocardial characterization. Indeed, inaccurate stroke volume estimation by Doppler echocardiography and inconsistent thresholds in current guidelines accounted for more than 40% of patients with discordant small-area, low-gradient aortic stenosis. These data may explain the variable prognosis reported in this unique group of patients, and argue for more accurate assessment of borderline cases with cardiovascular magnetic resonance.

Late gadolinium enhancement imaging detects focal areas of established myocardial fibrosis. In many conditions, including aortic stenosis, a more diffuse form of fibrosis predominates, which is potentially reversible and not readily identified by late gadolinium enhancement. Recently several myocardial T1 mapping approaches have been developed to quantify diffuse fibrosis. Using a standardized and systematic approach, I compared several commonly used T1 mapping techniques and identified that extracellular

volume had the best profile (reproducibility and discriminatory potential) for the identification of diffuse fibrosis in patients with aortic stenosis.

Cardiac troponin is a structural protein present in the cardiac myocytes. Recent advances in assay technology have substantially improved sensitivity, allowing quantification of troponin concentrations with a high degree of precision in everyone. In more than 250 patients with aortic stenosis, I demonstrated that cardiac troponin I concentrations were independently associated with markers of left ventricular decompensation (hypertrophy and fibrosis) and predicted clinical outcome in patients with aortic stenosis. This suggests that myocardial fibrosis detected by cardiovascular magnetic resonance is consequent on myocardial injury secondary to left ventricular decompensation.

Left ventricular hypertrophy with strain pattern on a 12-lead electrocardiogram is associated with poor outcome in patients with aortic stenosis, but the mechanism of this electrocardiographic pattern has not been described. In more than 300 patients with aortic stenosis, I demonstrated that these characteristic repolarization abnormalities were a highly specific marker of focal mid-wall myocardial fibrosis (specificity of 99% and sensitivity of 54%). Moreover, the prognostic value of this electrocardiographic pattern was again confirmed with markedly worse long-term outcomes in these patients.

CONCLUSION

I have demonstrated that cardiovascular magnetic resonance can assist in the assessment of disease severity in patients with aortic stenosis and discordant echocardiographic findings. Moreover, I have validated the assessment of diffuse myocardial fibrosis, as well as, demonstrated the close association between myocardial fibrosis and biomarkers of myocardial injury and electrocardiographic strain pattern that predicted an adverse outcome in patients with aortic stenosis.

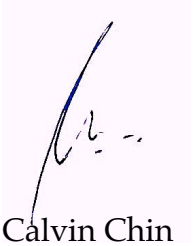
DECLARATION

This thesis represents the research I had performed at the Clinical Research Facility, Clinical Research Imaging Centre, and the Royal Infirmary of Edinburgh between March 2012 and December 2014.

I was personally involved in the recruitment and imaging of all the patients related to the study. I performed the echocardiograms, alongside a research ultrasonographer (Audrey White), supervised the cardiovascular magnetic resonance scans and had analyzed all the images and data presented in this thesis. Patients in the Outcome Cohort (outlined in Chapters 5 and 6) were recruited from the SALTIRE trial, conducted between March 2001 and April 2002. I had not recruited the patients, collected any data or performed any procedures in these patients.

Chapters 1, 3, 4, 5 and 6 have all been published in high-impact peer-reviewed medical journals. I was the first author for chapters 1, 3 and 4, and first co-authors for chapters 5 and 6. In chapters 5 and 6, I was responsible for recruitment of the patients and analysis of the data in the Mechanism Cohort. Dr Anoop Shah had specifically analyzed data in the Outcome Cohort in both chapters. We contributed equally to the drafting of the final manuscripts.

This thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged. The research was undertaken in accordance with the Declaration of Helsinki and the regulations of the South East Scotland Ethics Committee.



Calvin Chin March 4, 2015

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I would also like to thank Audrey White (Research Ultrasonographer) for her dedication and commitment in performing the echocardiograms, and the Clinical Research Facility at the Royal Infirmary of Edinburgh for providing us with the facilities to do so. In addition, I would like to thank Dr Sanjay Prasad and Dr Vassilis Vassiliou at the Royal Brompton Hospital for the wonderful collaboration and friendship. Indeed, I will look forward to more exciting collaborations with the University of Edinburgh and the Royal Brompton Hospital.

Last but not least, I extend my deepest gratitude to my wife Siska for her unceasing support, understanding and love during my PhD.

ABBREVIATIONS

σ	Myocardial wall stress
λ	Partition coefficient
AVA	Aortic valve area
BNP	Brain natriuretic peptide
CMR	Cardiovascular magnetic resonance
cTnI	Cardiac troponin I concentrations
DI	Dimensionless index
ECG	Electrocardiogram
ECV	Extracellular volume fraction
$LVOT_{area}$	Left ventricular outflow tract area
MPG	Mean pressure gradient
MOLLI	Modified look-locker inversion-recovery
NT-proBNP	N-terminal fragment proBNP
Z_{VA}	Valvulo-arterial impedance

CHAPTER 1

INTRODUCTION

Extracts from this chapter has been published in:

Chin CW, Vassiliou V, Jenkins WS, Prasad SK, Newby DE, Dweck MR. Markers of left ventricular decompensation in aortic stenosis. **Expert Rev Cardiovasc Ther**. 2014;12(7):901-912.

1.1 OVERVIEW

Calcific aortic stenosis is the most common valvular heart condition in developed countries, displaying an increasing prevalence with age (1). Aortic stenosis is characterized by progressive narrowing of the aortic valve, driven by a complex, active and highly regulated process of inflammation, fibrosis and calcification that leads to leaflet thickening and immobility (2,3). In response to the narrowed valve, left ventricular hypertrophy occurs initially to restore wall stress and cardiac performance, but ultimately this process decompensates and patients progress towards heart failure, symptoms and adverse clinical outcomes (4).

Contemporary guidelines advocate aortic valve replacement in patients with severe aortic stenosis and evidence of left ventricular decompensation defined by either the presence of symptoms or an impaired ejection fraction less than 50% (5,6). Echocardiography is the imaging modality of choice for assessing aortic stenosis: two dimensional imaging allows direct visualization of the valvular apparatus and cardiac chambers and Doppler techniques provide non-invasive assessment of haemodynamics (7). However, echocardiography is inherently limited, particularly in patients with poor acoustic windows and is more operator-dependent compared to other imaging modalities. Moreover, current approaches of assessing left ventricular decompensation also have crucial limitations. Aortic stenosis commonly occurs in elderly patients with comorbidities (such as coronary artery disease, hypertension and chronic lung diseases) that may confound symptom presentation and contribute to adverse cardiovascular outcomes. Importantly, an impaired ejection fraction occurs late in the disease process when myocardial damage may not be reversible. Whilst the risk of sudden cardiac death during the asymptomatic phase is relative low (~1% per year in large prospective series (8,9)), it is not negligible. Indeed, recent studies have suggested improved outcomes with early aortic valve replacement in asymptomatic patients with preserved systolic function (10,11).

A more thorough understanding of the mechanism of left ventricular decompensation is essential to identify patients with aortic stenosis who may benefit from early aortic valve replacement.

1.2 THE ASSESSMENT AND CLASSIFICATION OF AORTIC STENOSIS

The severity of aortic stenosis is classified using aortic valve area, mean transvalvular gradient and peak aortic jet velocity, with thresholds established from a variety of haemodynamic and natural history data (**Table 1.1**) (5-7).

TABLE 1.1. CLASSIFICATION OF AORTIC STENOSIS SEVERITY

Severity	Aortic Valve Area (cm^2)	Mean Pressure Gradient (mmHg)	Peak Aortic Jet Velocity (m/s)
Mild	> 1.5	< 20	2.0 to 2.9
Moderate	1.0 to 1.5	20 to 39	3.0 to 3.9
Severe	< 1.0	> 40	> 4

A normal aortic valve measured between 3.0 and 4.0 cm^2 and serious consequences occurred when valve area was reduced by more than 25% of the normal area; thus an aortic valve area of 1.0 cm^2 (corresponding to an indexed aortic valve area of 0.6 cm^2/m^2, assuming a body surface area of 1.75 m^2) was defined as severe aortic stenosis in adults (12). Although this threshold of 1.0 cm^2 was established from invasive cardiac catheterization, this was subsequently confirmed with valve areas estimated from the continuity equation and echocardiography (9,12,13).

Using the continuity equation, the calculated aortic valve area is based on the ratio between Doppler stroke volume and post-aortic valve flow. However, estimation of stroke volume by Doppler echocardiography assumes a circular and geometrically regular left ventricular outflow tract and a laminar flow profile, which is frequently not the case. In addition, the severity thresholds based on aortic valve areas and mean transvalvular gradients in current guidelines are inherently discordant: theoretical models have suggested an aortic valve area of 1.0 cm^2 corresponds more closely to a mean

transvalvular gradient of 30 to 35 mmHg, whilst the current recommended threshold of 40 mmHg corresponds to an aortic valve area of 0.8 to 0.9 cm^2 (14,15). The combination of these factors can have a significant impact on the assessment and classification of patients with aortic stenosis, particularly in patients with small aortic valve area, low transvalvular gradient aortic stenosis and preserved systolic ejection fraction (paradoxical low-flow low-gradient severe aortic stenosis). The outcomes associated with these patients have been variable in different studies, presumably reflecting a heterogeneous population and highlighting the limitations of current assessment and classification.

1.3 LEFT VENTRICULAR HYPERTROPHY: ADAPTATION TO HEART FAILURE

In conditions associated with left ventricular pressure overload such as aortic stenosis, myocyte size and myocardial wall thickness increase to restore wall stress (σ) according to the LaPlace's Law: $\sigma = [P \times r] / 2h$ where P is left ventricular pressure, r is left ventricular radius and h is the myocardial wall thickness. The changes in ventricular pressure, radius and wall thickness are initially adaptive, maintaining cardiac output and systolic function (16).

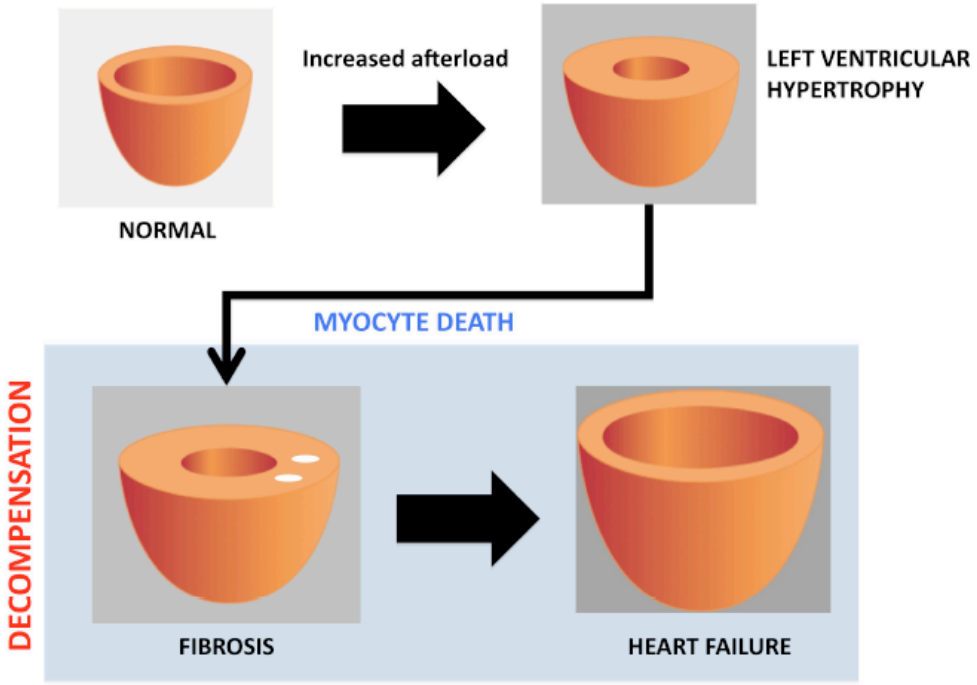
There is significant heterogeneity in the magnitude of hypertrophy that patients develop in response to similar degrees of aortic valve narrowing. Indeed, multiple studies have shown only a weak correlation between the severity of aortic valve narrowing and left ventricular mass: approximately 10 to 20% of patients with severe aortic stenosis have no evidence of left ventricular hypertrophy (17-22). Sex-related differences partially explain this variation, with women having smaller ventricles and lower myocardial mass compared to men (23-27), potentially as a consequence of differences in sex-related hormones and overall body mass (24,27). However, other clinical factors are also known to influence the magnitude of the hypertrophic response: age, the metabolic syndrome, obesity, ACE I/D polymorphisms, and importantly concomitant hypertension that imposes an additional load to the left ventricle (19,28-32). In order to account for both the arterial and valvular load on the left ventricle, a measure of the global left ventricular haemodynamic load (valvulo-arterial impedance, Z_{VA}) has been proposed, with Z_{VA} values >3.5 to 4.5 mmHg/mL/m^2 providing incremental prognostic value in patients with moderate and severe aortic stenosis (33,34).

As left ventricular hypertrophy increases, it will ultimately decompensate. This is characterized by progressive impairment in left ventricular performance and the development of symptoms (17,20,35) (**Figure 1.1**). The pathologic transition from ventricular adaptation to decompensation is driven primarily by two processes: myocyte death and myocardial fibrosis

(36). Myocyte death is predominantly in the form of proteosomal-mediated autophagy and oncosis (cellular and organelle swelling associated with increased membrane permeability), which occurs alongside more conventional forms of apoptosis. This cell death is believed to be activated by neurohumoral mediators such as angiotensin II and norepinephrine (37-40), and by progressive myocardial ischaemia. The latter relates to increased myocardial oxygen demand (due to the increased myocardial mass and afterload) and reduced coronary flow reserve (due to impaired microcirculatory perfusion and inadequate expansion of coronary capillary density despite the absence of coronary artery disease) (41,42).

Myocardial fibrosis is one of the histological hallmarks of end-stage heart failure (43,44). The pathogenesis of myocardial fibrosis is complex and the distribution varies, depending on the underlying pathology, although it generally exists in two predominant forms. **Replacement fibrosis** commonly occurs late in the disease process, is not believed to be reversible and is characterized by a more localized distribution corresponding to areas of myocyte loss. By contrast, **interstitial fibrosis** is more diffusely distributed, reflecting the more uniform and progressive accumulation of collagen in the interstitium, and is thought to be potentially reversible with targeted therapy (45-49). Both types of fibrosis are present in aortic stenosis, occupying up to 30% of the myocardium (16,50,51) and leading to progressive impairment of myocardial relaxation and contraction.

FIGURE 1.1. PATHOPHYSIOLOGY OF LEFT VENTRICULAR HYPERTROPHY AND THE TRANSITION TO HEART FAILURE IN AORTIC STENOSIS



In response to the narrowed aortic valve, left ventricular hypertrophy occurs initially to maintain cardiac output and wall stress. Ultimately it decompensates and heart failure and other symptoms ensue. The transition from adaptation to heart failure is driven by myocyte death and myocardial fibrosis, mediated by angiotensin II and norepinephrine activation as well as myocardial ischemia from increased afterload and left ventricular mass.

1.4 MARKERS OF LEFT VENTRICULAR HYPERTROPHY

Left ventricular hypertrophy is commonly assessed using the 12-lead electrocardiogram and echocardiography. By both methods, the presence of left ventricular hypertrophy in patients with aortic stenosis is associated with worse symptoms, impaired systolic function and an adverse prognosis (8,20,52,53). Data from the recent Simvastatin and Ezetimibe in Aortic Stenosis substudy reported an independent association between electrocardiographic left ventricular hypertrophy with strain and cardiovascular events in more than 1500 patients and 4 years of follow-up (8). However, the mechanism associated with this electrocardiographic strain was not known until recently. Electrocardiographic left ventricular hypertrophy with strain pattern was demonstrated to be a marker of an exaggerated hypertrophic response. Although these tests are non-invasive, inexpensive and well tolerated, an electrocardiogram is relatively insensitive in detecting left ventricular hypertrophy (54) and echocardiography relies heavily upon suitable acoustic windows, experience of the operator and a series of geometrical and mathematical assumptions (55). This may limit accurate measurements, particularly in subjects with distorted left ventricles or asymmetrical ventricular hypertrophy.

On the other hand, assessment by cardiovascular magnetic resonance is independent of geometric assumptions, providing highly accurate and reproducible measures of the left ventricle. Indeed, cardiovascular magnetic resonance is accepted as the non-invasive reference gold-standard for estimating left ventricular mass, volumes and ejection fraction (56,57) and it is being increasingly used to investigate the hypertrophic response in aortic stenosis. Consistent with previous electrocardiographic and echocardiographic data, indexed left ventricular mass assessed using cardiovascular magnetic resonance demonstrated an increased trend of predicting all-cause mortality in patients with moderate to severe aortic stenosis (58).

Traditionally, four patterns of ventricular hypertrophy based on myocardial wall thickness, left ventricular volume and mass have been described: normal geometry, concentric remodeling, concentric hypertrophy and eccentric hypertrophy (55). Recently, high spatial resolution cardiovascular magnetic resonance demonstrated the presence of asymmetric patterns of ventricular remodeling and hypertrophy in more than one in four patients with aortic stenosis (18). However, it remains unclear how patients transition between these different patterns, how they relate to the progression to heart failure and what the clinical consequences of these patterns might be.

Although the European Society of Cardiology had suggested aortic valve replacement be considered in patients with *excessive* left ventricular hypertrophy (Class IIb; Level of evidence: C) (5), the extent at which left ventricular hypertrophy is considered *excessive* in a patient is challenging to define. Instead, much of current evidence and ongoing research has focused on markers of ventricular decompensation secondary to maladaptive and advanced myocardial hypertrophy.

1.5 MARKERS OF DECOMPENSATION: LEFT VENTRICULAR PERFORMANCE

1.5.1 Systolic Function

Left ventricular ejection fraction is the conventional marker of global systolic dysfunction. Current guidelines recommend aortic valve replacement in patients with severe aortic stenosis and a reduced ejection fraction <50% (5,6). However, left ventricular ejection fraction is not sensitive to detect mild degrees of left ventricular systolic dysfunction (59,60). Moreover, the evidence for using ejection fraction as an indication for aortic valve replacement is weak. Indeed, this recommendation is largely based upon limited retrospective studies that demonstrated an improvement in left ventricular function following aortic valve replacement in patients with severe aortic stenosis and impaired ejection fraction (61,62).

Another limitation of using the ejection fraction is its tendency to overestimate myocardial systolic function in the presence of advanced concentric hypertrophy. This is because the associated increases in myocardial wall thickness and filling pressures, alongside reductions in ventricular volumes can result in a normal or even supra-normal ejection fraction despite significant impairment in intrinsic myocardial contractility (59,63,64). By contrast, echocardiographic assessment of mid-wall fractional shortening and longitudinal function better reflect such contractility. They have been associated with the presence of symptoms and the magnitude of the left ventricular afterload in aortic stenosis, although their prognostic significance remains to be established (65-68). In addition, novel myocardial deformation imaging (strain and strain rate) using two-dimensional speckle tracking echocardiography has been proposed as an alternative and highly sensitive technique for the assessment of intrinsic myocardial contractility (69,70). This approach measures the magnitude of myofibril contraction in the left ventricle, which varies in direction according to the different myocardial layers. Indeed, multi-directional strain imaging has demonstrated that myocardial dysfunction is present despite preserved

ejection fraction and interestingly, it progresses in a step-wise fashion from subendocardial dysfunction in mild aortic stenosis (abnormal longitudinal deformation), to mid-wall dysfunction in moderate aortic stenosis (abnormal circumferential deformation), and eventually transmural dysfunction in severe disease (abnormal radial deformation) (67,71,72). This technique also appears to provide prognostic information, with impaired longitudinal myocardial strain and strain rate predicting an adverse outcome in asymptomatic patients with aortic stenosis (34).

1.5.2 Diastolic Function

Impaired left ventricular relaxation occurs in aortic stenosis as a result of left ventricular hypertrophy and myocardial fibrosis (16,73-75), frequently preceding reductions in ejection fraction. Current studies examining diastolic dysfunction in aortic stenosis have largely relied on Doppler mitral inflow and myocardial tissue velocities (52,76-78), with limited data using myocardial strain and strain rate imaging. These echocardiographic measures of diastolic dysfunction are associated with worse symptomatic status (52,78), and predict adverse cardiovascular events (34,76,79). They therefore hold potential as early markers of left ventricular decompensation although their relationship with the more sensitive assessment of systolic dysfunction such as strain and strain rate imaging is not well understood and there is some inconsistency with respect to their prognostic value (77).

Measurement of the left atrial size is an alternative method for assessing diastolic function that has been the subject of several small-scale studies (34,79). It is also closely linked with the development of atrial fibrillation, which in the context of aortic stenosis is associated with advanced hypertrophy, an impaired ejection fraction and an increased risk of heart failure and cerebrovascular events (80).

1.5.3 Role of Exercise Stress Testing

The prompt identification of symptoms is crucial in the effective management of patients with aortic stenosis, given the poor prognosis associated with their development (4). However, it should be noted that the cardinal symptoms established by Ross and Braunwald (angina, exertional dyspnoea, pre-syncope and syncope) were based on young patients with bicuspid or rheumatic disease (average age of 63 years at time of death) compared to the older patients who present today with calcific aortic stenosis and comorbidities. The assessment of symptoms in contemporary clinical practice is therefore frequently challenging. Under reporting is common, and patients may unconsciously limit their activities to minimize symptoms. In these situations, exercise stress testing performed under close supervision and with careful monitoring of blood pressure and electrocardiographic changes may be helpful in unmasking otherwise latent symptoms. However, in a meta-analysis of 7 studies and 491 patients with asymptomatic severe aortic stenosis, the sensitivity, specificity, positive and negative predictive values for an adverse cardiac event after an abnormal exercise stress test were only modest at 75%, 71%, 66% and 79% respectively (81). Nevertheless, both the American Heart Association/American College of Cardiology and European Society of Cardiology guidelines recommend that aortic valve replacement be considered in patients who develop exercise-limiting symptoms or an abnormal blood pressure response (defined as an increase in systolic blood pressure of <20 mmHg) on exercise stress testing (5,6,82,83).

1.5.4 Brain Natriuretic Peptide

Interest has surrounded the use of the blood biomarkers brain natriuretic peptide (BNP) and the related N-terminal fragment of proBNP (NT-proBNP) in aortic stenosis. These are endogenous cardiac hormones released in response to increased left ventricular wall stress and are therefore elevated in patients with left ventricular dysfunction. Several studies have demonstrated that their levels increase as patients transition from hypertrophy to heart failure and that they hold promise in assessing patients with equivocal symptoms and severe disease (84-87).

The value of measuring BNP and NT-proBNP in patients who are asymptomatic is less certain. In many studies, BNP and NT-proBNP demonstrated a better association with clinical outcomes than traditional measures of aortic stenosis severity (88-91). However, two recent studies have questioned their prognostic value, failing to demonstrate an incremental prognostic value when other clinical and echocardiographic measures of aortic stenosis were also considered (86,92). Of note, patients in the latter two studies were older (79 to 83 years *versus* 68 to 74 years), hinting at an important limitation of these biomarkers. Both BNP and NT-proBNP increase substantially with advancing age independent of aortic valve disease (93,94) and this lack of specificity in the elderly (the population most commonly affected by aortic stenosis) makes the selection of appropriate thresholds difficult. Moreover, BNP and NT-proBNP lack sensitivity and levels only increase in the later stages of left ventricular decompensation when symptoms and other markers of left ventricular dysfunction are already apparent.

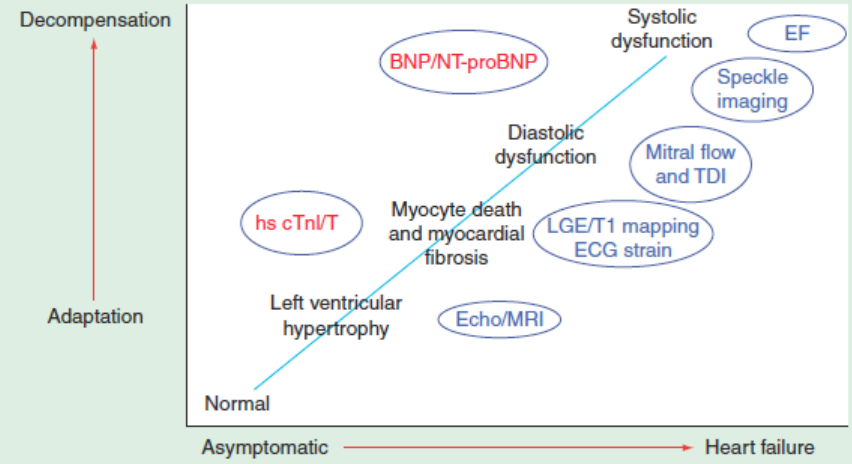
1.6 MARKERS OF DECOMPENSATION: MYOCARDIAL FIBROSIS

Myocardial fibrosis is one of the key mediators in the transition from compensatory left ventricular hypertrophy to heart failure, occurring early and crucially, before significant deterioration in cardiac diastolic/systolic function (50,95) (**Figure 1.2**). Therefore, there is considerable interest in novel biomarkers associated with this transition so that high-risk patients with aortic stenosis may be identified and offered prompt valvular replacement before heart failure ensues.

Myocardial biopsy remains the gold standard of diagnosing myocardial fibrosis. However, it is invasive, susceptible to sampling errors and unable to assess the fibrotic burden of the whole heart. Alternatively, echocardiography and circulating markers of collagen metabolism have been used as indirect measures of myocardial fibrosis but collagen markers lack specificity and integrated backscatter echocardiographic techniques are prone to artifacts (less than half of the patients have suitable backscatter signal for analysis) and have poor reproducibility (96-98).

Multiparametric cardiovascular magnetic resonance offers high-resolution whole-heart imaging and excellent myocardial characterization, providing a more accurate diagnosis than localized invasive myocardial biopsy. In addition, cardiovascular magnetic resonance allows serial assessment of myocardial remodeling and fibrosis over time. Currently, two approaches are used: late gadolinium enhancement, for direct visualization and quantification of focal replacement fibrosis, and novel myocardial T1 mapping, for assessing more diffuse patterns of myocardial fibrosis.

FIGURE 1.2. MARKERS OF LEFT VENTRICULAR HYPERTROPHY AND VENTRICULAR DECOMPENSATION IN AORTIC STENOSIS



	Marker	Clinical implications
Left ventricular hypertrophy	Increased left ventricular mass on echocardiography and cardiovascular magnetic resonance	Easy to measure. Patients with an advanced response are more likely to transition to heart failure and have an adverse prognosis
Myocyte death and myocardial fibrosis	High sensitivity cardiac troponins	Appears to be a sensitive marker of LV decompensation in aortic stenosis but not specific. Further studies are required to investigate its role in aortic stenosis
	Late gadolinium enhancement and myocardial T1 mapping	Replacement fibrosis assessed using late gadolinium enhancement imaging offers prognostic information in initial studies. It does not appear to reverse after aortic valve replacement. T1 mapping technique detects a different form of fibrosis that appears to occur earlier and is likely to be reversible
	Electrocardiographic left ventricular hypertrophy and strain	Cheap and widely available. It occurs late in the disease process and is associated with an advanced hypertrophic response and markers of LV decompensation. Offers important prognostic information
Diastolic dysfunction	Echocardiographic mitral inflow and tissue Doppler velocities	Easy to measure. However, there are conflicting results with respect to their ability to predict adverse events
Systolic dysfunction	Speckle imaging (strain and strain rate)	Holds potential in detecting early systolic dysfunction before changes in the ejection fraction are apparent
	Brain natriuretic peptide and N-terminal pro-BNP	Increased levels are not manifest until late in the disease process Helpful in patients with equivocal symptoms, but the prognostic value in asymptomatic patients is conflicting
	Ejection fraction	Underestimates intrinsic myocardial systolic dysfunction especially in presence of concentric hypertrophy

(**Abbreviations**: BNP brain natriuretic peptide; NT-proBNP N-terminal fragment of proBNP; ECG electrocardiogram; EF ejection fraction; LGE late gadolinium enhancement; TDI tissue Doppler imaging; hs cTnI/T high sensitivity cardiac troponin I/T; MRI cardiovascular magnetic resonance)

1.6.1 Late Gadolinium Enhancement

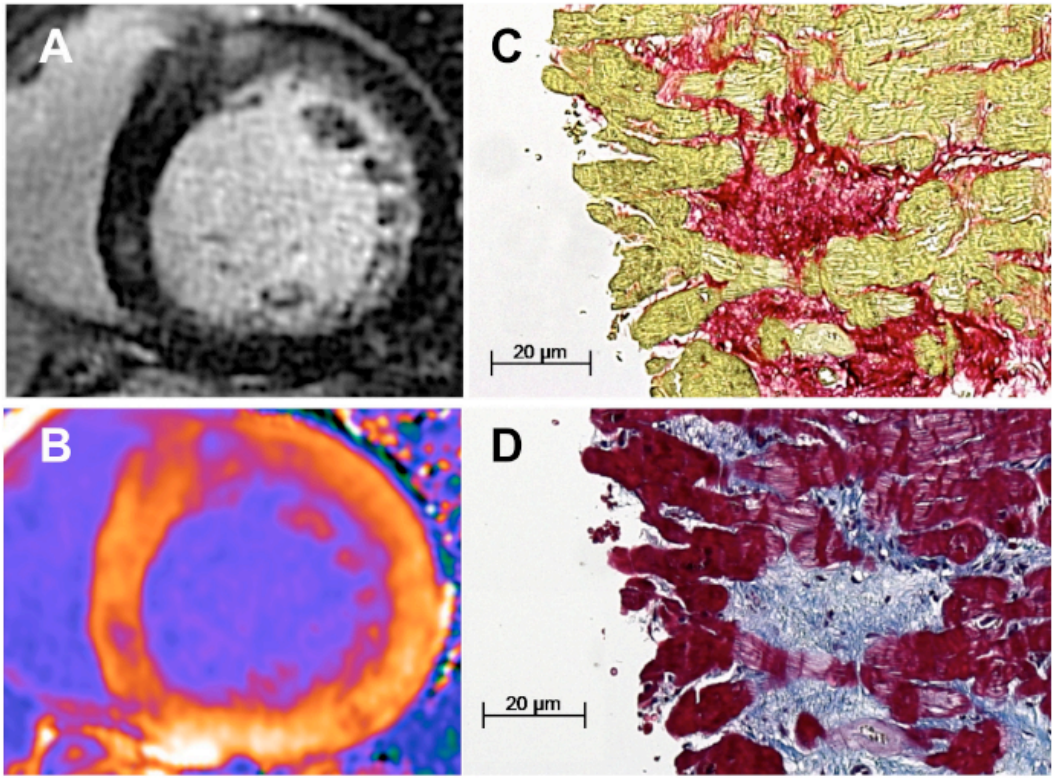
In cardiovascular magnetic resonance, the pixel signal intensity is based on the relaxation of hydrogen protons in a static magnetic field. One of the relaxation parameters is T1, the time constant (measured in milliseconds) corresponding to approximately 63% of its longitudinal recovery. T1 relaxation times depend on the underlying tissue composition: for example, T1 is shortest in fat and longest in water.

Extracellular gadolinium-based contrast agents distribute only in the intravascular and the extracellular space. After intravenous injection, gadolinium contrast diffuses into the extracellular space ('wash in'). As a result of redistribution and renal excretion, the blood concentration of gadolinium falls and the contrast is 'washed out' from the extracellular space into the blood pool. In the normal myocardium, contrast concentration in the extracellular space equilibrates rapidly with the blood pool. In regions of myocardial fibrosis, extracellular space is expanded because of excessive collagen deposition. As a result, gadolinium volume of distribution is increased and wash out is prolonged (99). The combination of these effects causes a significant difference in contrast concentration between normal and abnormal myocardium during the equilibrium phase following contrast administration.

In the presence of gadolinium-based contrast agents, T1 relaxation in regions of fibrosis is considerably shorter compared with surrounding normal myocardium. Using the conventional inversion-recovery gradient echo sequences, maximum difference in signal intensity between normal and abnormal myocardium is achieved at the "null" point – the inversion time where normal myocardium appears dark (100). This technique can therefore identify areas of replacement fibrosis in the myocardium which appear bright in the mid-wall of the left ventricle, in contrast to the surrounding black-appearing normal myocardium (**Figure 1.3**) (44,101,102). Importantly, this pattern of fibrosis can be differentiated from that observed with prior

myocardial infarction, which can also be observed in patients with aortic stenosis.

FIGURE 1.3. LATE GADOLINIUM ENHANCEMENT AND MYOCARDIAL T1 MAP



Late gadolinium enhancement imaging and myocardial T1 mapping in a patient with planned aortic valve replacement for severe aortic stenosis. Late gadolinium enhancement imaging demonstrates areas of replacement fibrosis in the basal antero- and infero-septal segments (A). 20 min post-contrast myocardial T1 map of the same basal slice reveals areas of replacement fibrosis, corresponding to the late gadolinium enhanced image (B). In addition, the extracellular volume fraction (ECV) calculated in this patient was elevated at 32.7% (the normal ECV in healthy volunteers was 26.0±1.6%). Myocardial biopsy sampled during aortic valve replacement confirms the presence of myocardial fibrosis. Collagen fibers stain pink with picrosirius red (C) and blue with Masson's trichrome (D).

Several studies have investigated the role of late gadolinium enhancement. A recent study of 143 patients with moderate to severe aortic stenosis demonstrated the presence of replacement fibrosis was an independent predictor of mortality, providing incremental prognostic value over and above that of the ejection fraction. Indeed, patients with myocardial fibrosis had an 8-fold increase in all-cause mortality compared to those without fibrosis despite similar aortic stenosis severity and coronary artery disease burden (58). Similar findings have also been observed in patients following aortic valve replacement, with the presence of replacement fibrosis being associated with adverse ventricular remodeling and worse peri-operative and long-term outcomes following aortic valve replacement (51,103-106).

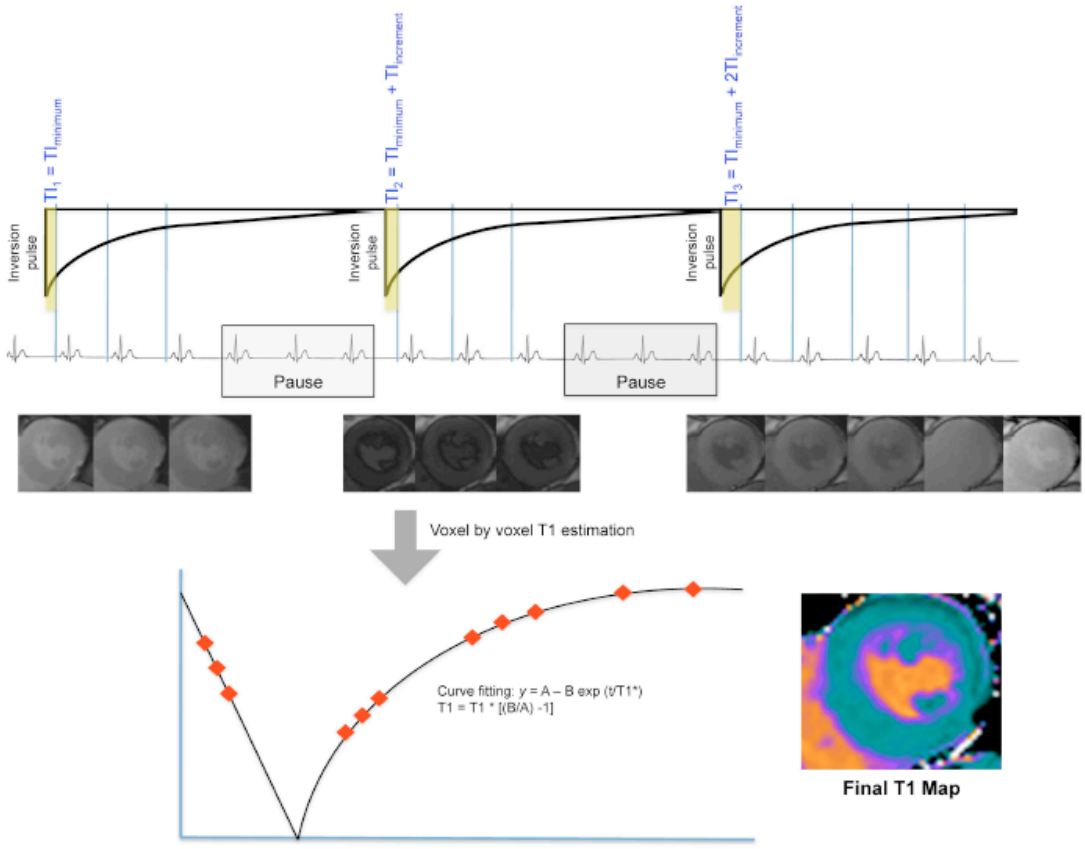
1.6.2 Myocardial T1 Mapping

As discussed earlier, the predominant form of myocardial fibrosis in aortic stenosis is actually interstitial not replacement fibrosis. As a consequence of its diffuse distribution, this form of fibrosis is not detected by late gadolinium enhancement, which relies on regional differences in signal intensity between normal and fibrotic regions (44). Instead, novel myocardial T1 mapping is an emerging technique to quantify this form of fibrosis (**Figure 1.3**) (107).

Current methods differ principally in magnetization preparation pulse sequences and image acquisition timings, with the Modified Look-Locker Inversion-recovery sequence being the most widely studied (107-110). The Modified Look-Locker Inversion-recovery sequence uses 17 heartbeats within one breath-hold to acquire 11 images with different inversion times during mid-diastole. Thereafter, the images were combined and the T1 in each individual voxel was estimated using a non-linear curve-fitting algorithm and a Look-Locker correction method (111) (**Figure 1.4**).

To date four major T1 measures have been assessed and validated against histology with promising results. The major strengths and limitations are discussed in **Table 1.2**. Although prospective outcome data associated with myocardial T1 mapping are lacking in aortic stenosis, they have been established in other cardiovascular patient populations (112-114).

FIGURE 1.4. MODIFIED LOOK-LOCKER INVERSION-RECOVERY SEQUENCE IMAGE ACQUISITION



After the inversion pulse, the magnetization following the T1 relaxation curve is repetitively sampled in several heartbeats until longitudinal mangetization has fully recovered. In this case, a **3-3-5** heart beat acquisition scheme is used: images were acquired consecutively for 3, 3 and 5 heart beats with a 3 beat pause in between. By combining several inversions with slightly shifted inversion times (TI), the relaxation curve can be sampled within a single breath-hold. Thereafter, the T1 is estimated on a voxel by voxel basis using a non-linear curve fitting algorithm and Look-Locker correction method to create the final T1 map.

TABLE 1.2

DESCRIPTION OF COMMONLY USED T1 MEASURES IN THE ASSESSMENT OF INTERSTITIAL FIBROSIS

T1 Measure	Characteristics	Advantages	Limitations
Native (non-contrast) T1	Native myocardial T1 values are higher in areas of fibrosis	No contrast required	It measures a composite of both interstitial and myocyte T1, thus it may not be sensitive in less severe myocardial fibrosis
Post-contrast T1	Gadolinium accumulates in areas of fibrosis because of an expanded extracellular volume Post-contrast T1 values are reduced in areas of fibrosis	Contrast improves sensitivity in identifying myocardial fibrosis Can be incorporated in clinical scan easily	Confounded by individual variation in gadolinium kinetics, and timing of imaging Poor scan-rescan reproducibility
Partition coefficient (λ)	Estimates contrast volume of distribution in the interstitial space Expressed as a ratio of T1 signal change in the myocardium and blood pool $\lambda = \Delta R_{myocardium} / \Delta R_{blood\ pool}$, where ΔR = 1/post-contrast T1 – 1/native T1	Excellent scan-rescan reproducibility	Does not account for contrast volume of distribution in plasma
Extracellular volume fraction (ECV)	Similar to partition coefficient, corrects for contrast volume of distribution in the plasma ECV = λ * [1-hematocrit]	Excellent scan-rescan reproducibility	Hematocrit sampling required in patients Comparison of values across centers may be limited by variabilities in scanners and protocols

1.6.3 High-sensitivity Cardiac Troponin

A plasma biomarker that appears to be released early during the transition from hypertrophy to heart failure is cardiac troponin. Increased cardiac troponin concentrations have traditionally been considered to be a highly specific marker of myocardial necrosis in patients with acute coronary syndromes (115). However recent advances in assay sensitivity allow quantification of plasma cardiac troponin with a high degree of precision at extremely low plasma concentrations (116). This allows the detection of myocardial injury in a wide range of cardiac conditions aside from acute coronary syndromes, including aortic stenosis. As previously discussed, myocyte death is one of the key factors driving left ventricular decompensation in aortic stenosis, and this provides a clear rationale for troponin as a cheap and potentially widely available biomarker of this process.

In a recent study, high-sensitivity plasma cardiac troponin T concentrations were detectable in all 57 patients with moderate and severe aortic stenosis. Moreover, these concentrations correlated positively with left ventricular wall thickness, ventricular mass and the severity of aortic stenosis but interestingly did not appear related to concomitant coronary artery disease. Furthermore, the highest quartile of high-sensitivity troponin T concentrations was associated with worst 2-year survival rates (117). Although early data is encouraging, larger studies are needed to investigate the potential clinical role for high-sensitivity cardiac troponin and the mechanism of its release in aortic stenosis.

1.7 THESIS AIMS AND HYPOTHESES

The aims of the thesis are:

1. To compare stroke volume estimation using echocardiography with the standard reference of using cardiovascular magnetic resonance and establish the optimal threshold for severe aortic stenosis

2. To characterize the temporal and regional T1 profiles of the myocardium and to identify the optimal approach based upon its reproducibility and ability to differentiate asymptomatic patients with aortic stenosis from healthy volunteers

3. Using a high-sensitivity assay and cardiovascular magnetic resonance, to establish the determinants and long-term prognosis associated with plasma cardiac troponin I concentrations in patients with aortic stenosis

4. To investigate the association between electrocardiographic strain pattern and extent of ventricular hypertrophy and myocardial fibrosis assessed using cardiovascular magnetic resonance; and to examine the prognosis associated with this electrocardiographic pattern in patients with aortic stenosis

The hypotheses of the thesis are:

1. Combination of left ventricular outflow tract area underestimation and inconsistent thresholds influence the classification of aortic stenosis severity, and contribute to patients with small aortic valve area, low transvalvular gradient aortic stenosis (Chapter 3)

2. Among the commonly used T1 measures, extracellular volume fraction has the best profile (reproducibility and ability to differentiate patients with aortic stenosis from healthy volunteers) and therefore, holds the most potential to assess diffuse myocardial fibrosis in aortic stenosis (Chapter 4)

3. Detection of myocardial injury by high-sensitivity troponin assays provide an early indication of left ventricular decompensation and it is associated with long-term cardiovascular events in patients with aortic stenosis (Chapter 5)

4. Left ventricular hypertrophy with strain on an electrocardiogram is a marker of left ventricular decompensation and it predicts adverse cardiac events in patients with aortic stenosis (Chapter 6)

CHAPTER 2

METHODOLOGY

2.1 PATIENT POPULATION

Stable adults with mild to severe aortic stenosis (including those with planned aortic valve replacement) and who were able to provide informed consent were eligible for the studies. These patients were prospectively recruited from the Edinburgh Heart Centre, United Kingdom. The **exclusion criteria** were: (1) other significant valvular heart disease (defined as moderate or worse in severity); (2) significant co-morbidities with limited life expectancy (such as advanced malignancy, end-stage heart failure); (3) contraindications for cardiovascular magnetic resonance such as implantable cardiac devices, renal impairment with glomerular filtration rate <30 mL/min/1.73 m^2, ocular metallic foreign bodies, cranial aneurysmal clips and women who were pregnant or lactating; (4) acquired or inherited cardiomyopathies (including previous myocarditis). The presence of coronary artery disease was defined by previous infarction, clinical symptoms of angina (in those with mild or moderate aortic stenosis), evidence of myocardial ischemia or >50% luminal stenosis in a major epicardial vessel.

Written informed consent was obtained from all participants. All studies had received ethical approval from the South East Scotland Research Ethics Committee and the research was conducted in accordance with the Declaration of Helsinki. Where appropriate, studies were registered with clinicaltrials.gov (NCT01755936).

2.2 ECHOCARDIOGRAPHY

Comprehensive transthoracic echocardiography was performed in all patients (iE33, Philips Medical Systems, Best, the Netherlands) by a research ultrasonographer (Audrey White) and a cardiologist certified in echocardiography (Dr Calvin Chin). The severity of aortic stenosis was assessed and classified according to the European Association of Echocardiography/American Society of Echocardiography guidelines (7).

Left ventricular outflow tract diameter was measured in the parasternal long-axis view, at the insertion of the aortic cusps from the inner edge of the septal endocardium to the inner edge of the anterior mitral leaflet in mid-systole. Left ventricular outflow tract velocity-time integral was measured in the apical 5-chamber view using pulsed-wave Doppler just proximal to the aortic valve, careful to obtain a laminar spectral tracing to avoid contamination from flow across the aortic valve. The peak aortic jet velocity and mean transvalvular gradient were derived from the aortic valve velocity-time integral, using continuous-wave Doppler. The highest aortic jet velocity and mean transvalvular gradient were determined in multiple acoustic windows using standard S51 and D2cwc probes (Philips Medical Systems, Best, the Netherlands), and corroborated by the 2 operators. The mean of 3 readings (5 if the patient had atrial fibrillation) was recorded. Aortic valve area was calculated with the continuity equation (Aortic valve area = Doppler stroke volume/aortic valve velocity-time integral; Doppler stroke volume = left ventricular outflow tract area x left ventricular outflow tract velocity-time integral). In our centre, we were able to achieve excellent reproducibility in the assessment of aortic stenosis severity (intra- and inter-observer reproducibility of 4.9% and 6.9% for aortic valve area, respectively) (118).

Diastolic function was determined using the standard measures (119). Transmitral early and late diastolic velocities and deceleration time of early filling velocity were measured at the tips of the mitral valve leaflets using pulsed-wave Doppler. The mean early diastolic velocities of the medial and lateral mitral annulus were measured using pulsed-wave tissue Doppler imaging.

2.3 CARDIOVASCULAR MAGNETIC RESONANCE

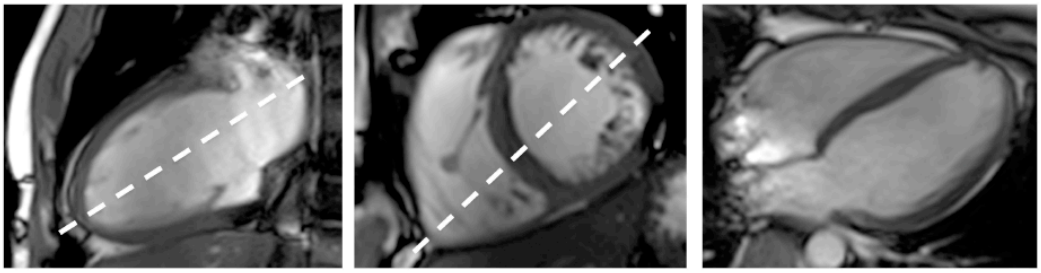
Cardiovascular magnetic resonance was performed at 3T (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany) according to a standardized protocol. Localizer scans were first performed to ascertain the anatomical position of the heart and to plan subsequent examinations. Rapid axial views of the entire thorax were performed with an ultra fast spin echo sequence in a single breath hold (Half-fourier Acquisition Single-shot Turbo Spin Echo; HASTE). Double-oblique sections of the heart were performed with a high-resolution balanced steady-state free precision sequence (True Fast-Imaging with Steady state Precision; TrueFISP) to obtain the standard long axis two-chamber, three-chamber, four-chamber and axial view of the aortic valve (**FIGURE 2.1**). Short-axis cine images extending from the mitral valve to the left ventricular apex (8 mm parallel slices with 2 mm spacing; temporal resolution ≤ 45 ms) were used for the assessment of left ventricular volumes, mass and function.

In this study, diffuse myocardial fibrosis is assessed using myocardial T1 mapping, performed using the Modified Look-Locker Inversion-recovery (flip angle 35°; minimum TI 100 ms; TI increment of 80 ms; time delay of 150 ms; heart beat acquisition scheme of 3-3-5) with built-in motion correction (109,110,120). A gradient echo field map and associated shim were performed to minimize off-resonance frequency artifact. Short axis T1 maps of the basal, mid-cavity and apical slices were acquired before and at 20 min following the administration of 0.1 mmol/kg of gadobutrol (Gadovist, Bayer Pharma AG, Germany). The basal slice was defined as the first complete ring of myocardium below the left ventricular outflow tract, and the mid-cavity slice as the most basal slice to include both papillary muscles. The apical slice was selected between the apex and the mid-cavity on the image least affected by trabeculations and partial volume averaging. The acquisition sequence and the commonly used T1 measures have been described in detail in Chapter 1. In a recent study, we had compared the commonly used T1 measures and demonstrated pre- and post-contrast T1 had excellent intra-

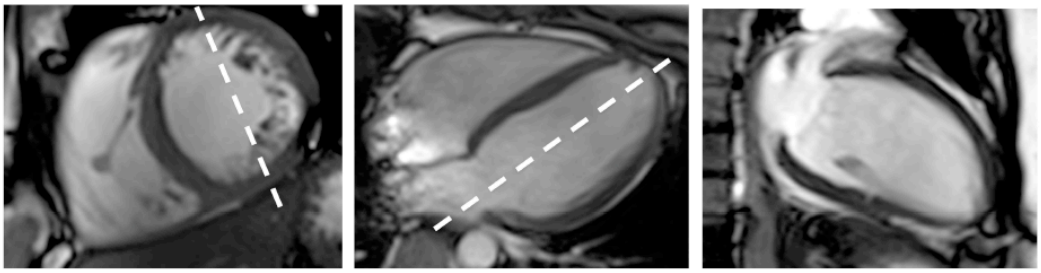
and inter-observer reproducibility (intra-class coefficient >0.95) and good scan-rescan reproducibility (intra-class coefficient 0.56 to 0.72). On the other hand, both partition coefficient and extracellular volume fraction had the best reproducibility profile (intra-class coefficient of >0.95 and a variability of < 2.5% for inter-, intra-observer and scan-rescan reproducibilities) (121).

Late gadolinium enhancement was performed between 8 and 15 min following gadobutrol administration. Two approaches were used: an inversion-recovery fast gradient-echo sequence and a phase-sensitive inversion-recovery sequence in two phase-encoding directions to differentiate true enhancement from artifact (100,122). The inversion time for the inversion-recovery fast gradient-echo sequence was optimized for each slice to achieve satisfactory nulling of the myocardium.

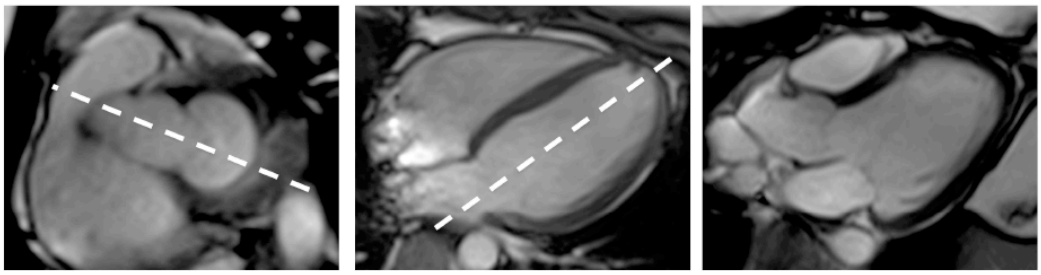
FIGURE 2.1. PLANNING THE BASIC CARDIAC VIEWS



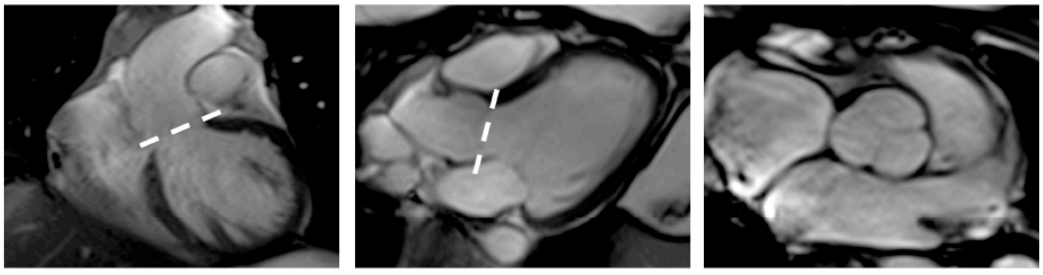
Four Chamber Cine: Prescribe a slice bisecting the left ventricle through the mitral valve and apex on a two-chamber view (left) and a slice bisecting the left and right ventricles on a short axis view (middle)



Two Chamber Cine: Prescribe a slice parallel to the septum, through the anterior and inferior walls on a short axis (right) and a slice bisecting the left ventricle through the mitral valve and apex on a four-chamber view (middle)



Three Chamber Cine: Prescribe a slice bisecting the left ventricular outflow tract and inferolateral wall on the most basal short axis slice (left) and 1 slice bisecting the left ventricle through the mitral valve and apex on a four-chamber view (middle)



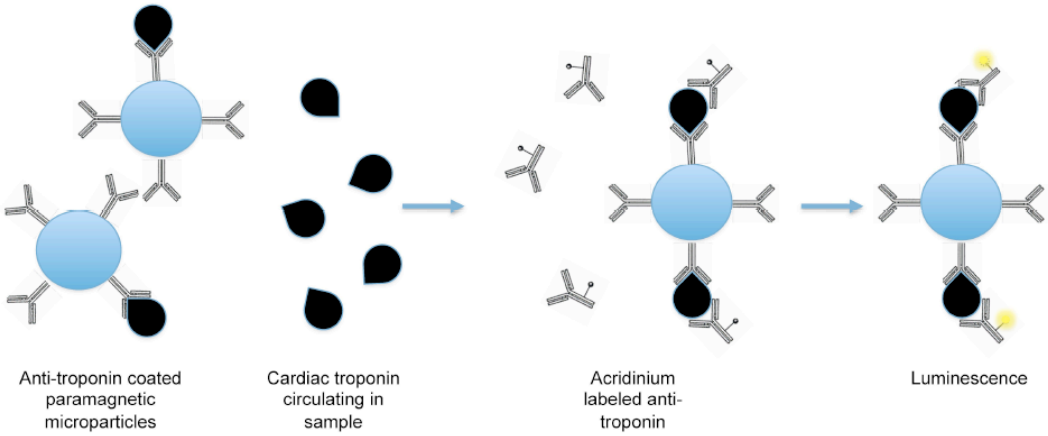
Aortic Valve Cine: Prescribe a slice perpendicular to the aortic annulus in the aortic outflow (left) and three-chamber (middle) views. Three contiguous cross-sectional slices are performed to image the aortic valve at its tips

2.4 HIGH SENSITIVITY CARDIAC TROPONIN I

Plasma cardiac troponin I concentrations were determined using a high-sensitivity assay (ARCHITECT *STAT*, Abbott Laboratories, Abbott Park, IL, USA). The ARCHITECT *STAT* assay uses the chemiluminescent microparticle immunoassay technology. In the first step, the sample and anti-troponin I antibody coated paramagnetic microparticles are combined. Cardiac troponin I present in the sample binds to the anti-troponin I coated microparticles. After washing, the acridinium-labeled anti-troponin conjugate is added in the second step. Following another wash, pre-trigger and trigger solutions are added to the reaction, resulting in a chemiluminescent reaction (**Figure 2.2**). This reaction is measured in relative light units by the ARCHITECT *i* System optics and the cardiac troponin I concentration is reported based on a standard calibration curve established with known concentrations (123).

The lower limit of detection of this assay is 1.2 ng/L (116) and the 99th centile from a healthy reference population is 26 ng/L, with a 10% inter-assay coefficient of variation at 4.7 ng/L (123). Precision profiling of the assay was also performed in our centre (248 samples in 18 healthy controls). The inter-assay coefficient of variation for duplicate samples was 10% at 6 ng/L and 20% at 1.5 ng/L.

FIGURE 2.2. BIOLOGICAL PRINCIPLES OF THE HIGH-SENSITIVITY TROPONIN I ASSAY



The ARCHITECT *STAT* high-sensitivity troponin I assay is a two-step immunoassay that uses the chemiluminescent microparticle immunoassay technology for the quantification of cardiac troponin I in human plasma and serum. The resulting chemiluminescent reaction is measured in relative light units. A direct relationship exists between the relative light units and troponin I concentrations, and these concentrations can be established based on a known calibration curve.

2.5 ELECTROCARDIOGRAM

In all patients, a standard resting 12-lead electrocardiogram (10 mm/mV at 25 mm/s) was acquired in supine position using the same machine (Philips Pagewriter TC50, Philips Medical Systems, Massachusetts, USA). Electrocardiograms were performed on the day of cardiovascular magnetic resonance imaging, and interpreted according to the recommendations by the American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society (124). Poor quality electrocardiograms were repeated. Electrocardiographic left ventricular hypertrophy was diagnosed using the Romhilt-Estes system (score ≥5) (125) and QRS duration, PR and QT intervals were determined based on the Philips DXL ECG Algorithm (126).

2.6 COMPUTED TOMOGRAPHY

Patients in the Outcome Cohort (Chapters 5 and 6) also underwent computed tomography of the aortic valve. This cohort consisted of patients from the Scottish Aortic Stenosis Lipid Lowering, Impact on Regression (SALTIRE) and the imaging methodology had been described previously (127). Computed tomography was performed using a double-helix scanner (Twin II Flash, Philips Medical Systems, Massachusetts, USA). The aortic valve was scanned with 2.7-mm slices, increments of 1.4 mm during inspiratory breath-holding sessions. The images were analysed by a single operator using an automated software (Picker Cardiac Scoring), involving a modified Agatston scoring method with a threshold of 90 Hounsfield units to compensate for non-gated imaging (127). Computed tomography aortic valve calcium score had an excellent reproducibility of 0.07 log arbitrary units (128).

2.7 IMAGE ANALYSIS

The quantification of left ventricular volumes, function and mass was performed using the Argus Ventricular Function software (Siemens AG Healthcare Sector, Erlangen, Germany). Papillary muscles and minor trabeculations were included in the volume measurements (and excluded in left ventricular mass measurements) during both phases of the cardiac cycle, indexed to body surface area. Normal indexed volumes, ejection fraction were defined using sex- and age-specific ranges (129). Left ventricular mass was calculated from the total end-diastolic myocardial volume multiplied by the specific gravity of the myocardium (1.05 g/mL).

The presence of myocardial enhancement was independently determined by two experienced operators (Dr Calvin Chin and Dr Marc Dweck). The extent of mid-wall late gadolinium enhancement was quantified using QMASS software (Medis medical Imaging Systems, Leiden, the Netherlands) using a signal intensity threshold of > 2 standard deviations above the mean value in an adjacent normal region of myocardium. Areas of inversion artifact, or contamination by blood pool or epicardial fat, were excluded.

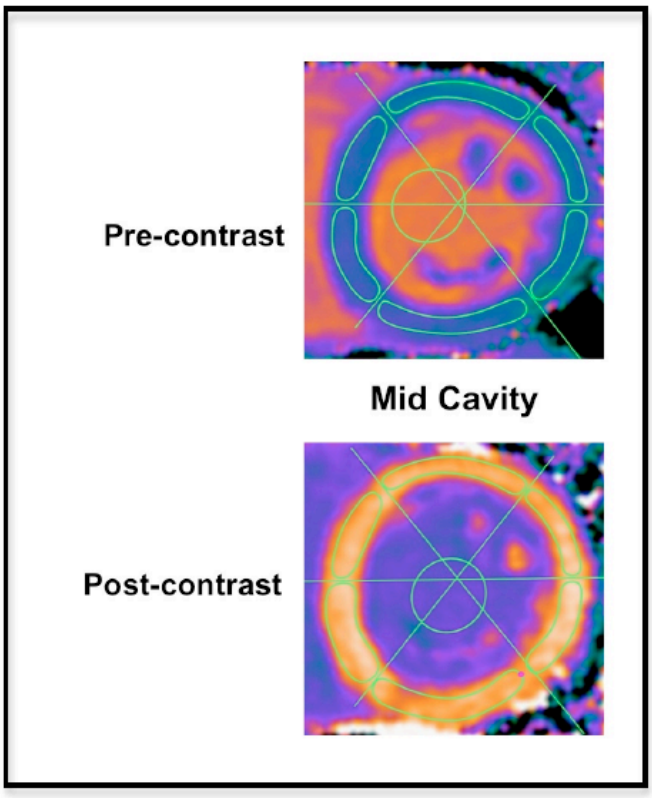
The quality of T1 map data was examined using the individual inversion recovery images. All segments affected by off-resonance, excessive breathing motion artifacts not corrected by the inline motion correction, and mistriggering were excluded from the analysis. To minimize partial volume effects from surrounding tissues and blood pool, we standardized the windowing and placement of regions of interest around the mid-cavity myocardium using a pre-defined protocol (121). The regions of interest were first drawn on the short-axis pre-contrast motion-corrected myocardial T1 maps and then copied onto each of the corresponding 20 min post-contrast T1 maps with stringent adjustments applied to avoid blood pool and artifacts (OsiriX version 4.1.1, Geneva, Switzerland) (**Figure 2.3**). Extracellular volume fraction (ECV) values were calculated:

$$\lambda = \Delta R1_{myocardium} / \Delta R1_{blood\text{-}pool}, \text{ where } R1=1/T1 \quad [1]$$

$$ECV = (1\text{-haematocrit}) \times \lambda \quad [2]$$

Haematocrit was determined at the time of cardiovascular magnetic resonance.

FIGURE 2.3. METHOD OF ASSESSING MYOCARDIAL T1



Regions of interest were drawn within the borders on the pre-contrast myocardial T1 maps and then copied onto the corresponding post-contrast images. Minor adjustments were made to avoid artifact and blood pool. A separate region of interest was also drawn in the left ventricular blood pool in order to calculate the extracellular volume fraction.

2.8 SAMPLE SIZE CALCULATIONS AND STATISTICAL ANALYSIS

From our previous study, the all-cause mortality rates (regardless of aortic valve replacement) in patients with and without mid-wall fibrosis were 143/1000 patient-years and 15.7/1000 patient-years, respectively (58). A sample size of 150 patients with aortic stenosis would be needed to detect an absolute survival rate difference of 13% with a power of 0.80 and a two-sided Type I error of 0.05. The prevalence of mid-wall fibrosis was assumed to be about 30% (58).

The distribution of all continuous variables was assessed for normality using the Shapiro-Wilk test. Continuous variables were presented as mean ± standard deviation or median [inter-quartile range] as appropriate. Comparison for normally distributed data was performed using Student's *t*-test or analysis of variance with *post hoc* Bonferroni adjustment. The Mann-Whitney *U* test and Kruskal-Wallis with *post hoc* Dunn tests were used for non-parametric data comparisons. Categorical variables were expressed as percentages and compared using the χ^2 test. The correlation between continuous data was assessed with the Pearson correlation.

Standard statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA) and SPSS Version 19 (SPSS, Inc., Chicago, IL, USA), unless otherwise stated. A two-sided $P < 0.05$ was considered statistically significant.

CHAPTER 3

ECHOCARDIOGRAPHY UNDERESTIMATES STROKE VOLUME AND AORTIC VALVE AREA

IMPLICATIONS FOR PATIENTS WITH SMALL-AREA LOW-GRADIENT AORTIC STENOSIS

Published in:

Chin CW, Khaw HJ, Luo E, Tan S, White AC, Newby DE, Dweck MR. Echocardiography underestimates stroke volume and aortic valve area: implications for patients with small-area low-gradient aortic stenosis. **Can J Cardiol.** 2014;30(9):1064-1072.

3.1 SUMMARY

AIMS: Discordance between small aortic valve area (AVA, <1.0 cm^2) and low mean pressure gradient (MPG, <40 mmHg) affects a third of patients with moderate or severe aortic stenosis. We hypothesized that this is largely due to inaccurate echocardiographic measurements of the left ventricular outflow tract area ($LVOT_{area}$) and stroke volume alongside inconsistencies in recommended thresholds.

METHODS: 133 patients with mild to severe aortic stenosis and 33 control individuals underwent comprehensive echocardiography and cardiovascular magnetic resonance (CMR). Stroke volume and $LVOT_{area}$ were calculated using both echocardiography and CMR, and the effects on AVA estimation assessed. The relationship between AVA and MPG measurements was then modeled with non-linear regression and consistent thresholds for these parameters calculated. Finally the impact of these modified AVA measurements and novel thresholds on the number of patients with small-area low-gradient aortic stenosis was investigated.

RESULTS: Compared to CMR, echocardiography underestimated $LVOT_{area}$ (n=40, -0.7 [95%CI -2.6 to 1.3] cm^2), stroke volumes (-6.5 [95%CI -28.9 to 16.0] mL/m^2) and consequently, AVA (-0.23 [95%CI -1.01 to 0.59] cm^2). Moreover an AVA of 1.0 cm^2 corresponded to MPG of 24 mmHg based on echocardiographic measurements and 37 mmHg after correction with CMR-derived stroke volumes. Based on conventional measures, 56 patients had discordant small-area low-gradient AS. Using CMR-derived stroke volumes and the revised thresholds, a 48% reduction in discordance was observed (n=29).

CONCLUSIONS: Compared to CMR, echocardiography underestimates $LVOT_{area}$, stroke volume and therefore AVA. The thresholds based on current guidelines are also inconsistent. The combination of these factors explain >40% of patients with discordant small-area low-gradient aortic stenosis.

3.2 INTRODUCTION

Discordant small aortic valve area (small-area; aortic valve area <1.0 cm^2), low mean pressure gradient (low-gradient; mean pressure gradient <40 mmHg) aortic stenosis occurs in about 30% of patients with aortic stenosis evaluated using echocardiography (130,131). This has classically been attributed to patients with low flow states, such as those with reduced left ventricular ejection fractions (7). However, in recent years, it has been recognized that small-area low-gradient aortic stenosis can also be observed in the presence of a preserved ejection fraction: so-called "paradoxical low-flow, low-gradient severe aortic stenosis". The outcomes associated with such patients have been variable in different studies (132-135), presumably reflecting a heterogeneous population and highlighting the uncertainty with regards to the actual severity of aortic stenosis in this subgroup.

Using the continuity equation, the aortic valve area is calculated based upon the ratio between the Doppler stroke volume and the post-aortic valve flow. Doppler stroke volume relies crucially on accurate estimation of the left ventricular outflow tract area ($LVOT_{area}$) according to the formula: Doppler stroke volume = $LVOT_{area}$ x LVOT flow. On two-dimensional echocardiography the $LVOT_{area}$ is derived from left ventricular outflow tract diameter measurements made on the parasternal long-axis view and the assumption that the left ventricular outflow tract is circular. However recent experience from transcatheter aortic valve replacement sizing has demonstrated that the left ventricular outflow tract is frequently elliptical not circular and as a consequence, measurements made by echocardiography underestimate the true $LVOT_{area}$ (136,137). The implication is therefore that echocardiography might also underestimate the left ventricular stroke volume and aortic valve area.

In addition, it is widely acknowledged that the severity thresholds for aortic valve area and mean transvalvular gradient recommended by current guidelines are inherently inconsistent (15,130), with theoretical models

suggesting an aortic valve area of 1.0 cm^2 corresponds more closely to a mean pressure gradient of 30 to 35 mmHg than the recommended threshold of 40 mmHg (14,15).

We hypothesized that the combination of $LVOT_{area}$ underestimation and inconsistent thresholds might influence the classification of aortic stenosis severity, and contribute to the number of patients with discordant small-area low-gradient aortic stenosis. The aims of the study were firstly to compare stroke volume estimation by echocardiography with the gold-standard non-invasive cardiovascular magnetic resonance (CMR) assessment and to establish the optimal thresholds for severe aortic stenosis. Subsequently, we then sought to investigate whether correcting for these two factors might impact on the number of patients with discordant small-area low-gradient aortic stenosis.

3.3 METHODS

3.3.1 Study Population

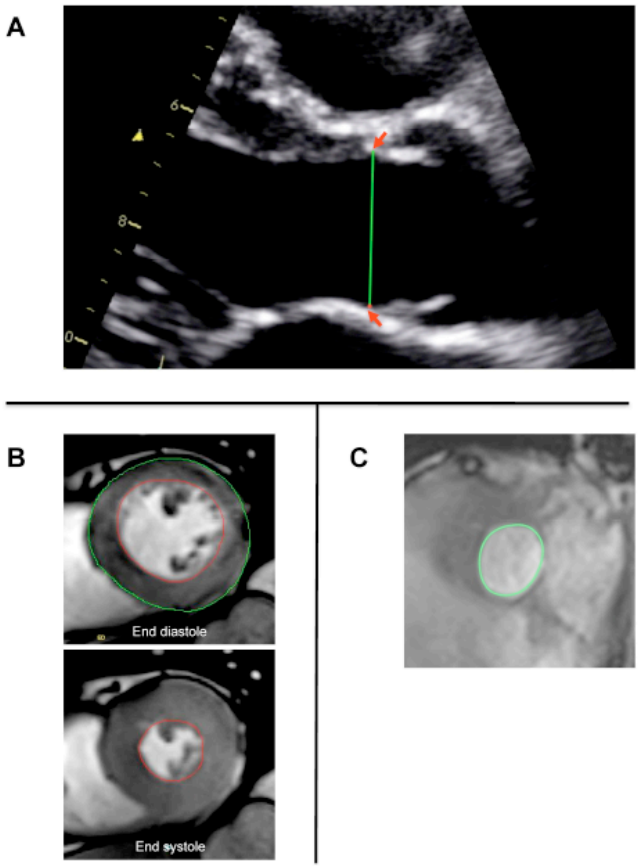
Patients with mild to severe aortic stenosis were prospectively recruited from the Edinburgh Heart Centre. The exclusion criteria have been described in Chapter 2. In addition, control individuals without aortic stenosis were recruited from the local community.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the local ethics committee. Written informed consent was obtained in all subjects.

3.3.2 Echocardiography

Standard echocardiographic techniques to assess severity of aortic stenosis have been carefully described in Chapter 2. Specifically, the left ventricular outflow diameter was measured at the insertion of the aortic cusps, from the inner edge of the septal endocardium to the inner edge of the anterior mitral leaflet in mid-systole (**Figure 3.1A**), because the cross-sectional shape is believed to more circular at this level (7). Doppler stroke volume was estimated ($LVOT_{area}$ x left ventricular outflow tract velocity-time integral) and used to calculate the AVA with the continuity equation (stroke volume/aortic valve velocity-time integral). Normal stroke volume by echocardiography was defined as ≥35 mL/m^2 (138). In a further analysis, we had also estimated stroke volume according to the Teichholz method (139) and the effects on aortic stenosis classification. In addition, the severity of aortic valve calcification was assessed in the short-axis view of the aortic valve using a score of 1 to 4 (140), and corroborated between the two operators. Valvuloarterial impedance, a measure of global afterload, was calculated as [systolic blood pressure + mean pressure gradient]/CMR stroke volume.

FIGURE 3.1. ESTIMATION OF THE LEFT VENTRICULAR OUTFLOW TRACT (LVOT) AREA USING ECHOCARDIOGRAPHY AND CARDIOVASCULAR MAGNETIC RESONANCE



(**A**) The LVOT diameter was measured at the aortic cusp insertion points (**red arrows**) in the parasternal long axis view. The LVOT area was estimated from the diameter measured. (**B**) The stroke volume was calculated as the difference between end-diastolic and end-systolic volumes. Planimetry of the endocardial borders (**red contours** in end-diastolic and end-systolic frames) was performed including the papillary muscles and minor trabeculations in volume measurements during both phases of the cardiac cycle. Left ventricular mass was calculated by multiplying the total end-diastolic myocardial volumes (**green and red contours** in the end-diastolic frame) by the specific gravity of the myocardium (1.05 g/mL). Papillary muscles and minor trabeculations were excluded in mass measurements, with care taken to avoid right ventricular trabeculations. (**C**) Planimetry of the LVOT area in the coaxial short axis view on cardiovascular magnetic resonance imaging at mid-systole.

3.3.3 Cardiovascular Magnetic Resonance

All participants underwent cardiovascular magnetic resonance at 3T (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany) according to the protocol specified in Chapter 2. Papillary muscles and minor trabeculations were included in the volume measurements during both phases of the cardiac cycle (**Figure 3.1B**) and stroke volume was measured as the difference between the end-diastolic and end-systolic left ventricular volumes (given the absence of significant mitral regurgitation), indexed to body surface area. Normal indexed left ventricular volumes, stroke volumes and ejection function were defined using sex- and age-specific ranges (129). Left ventricular mass was calculated from the total end-diastolic myocardial volume (excluding papillary muscles and minor trabeculations) multiplied by the specific gravity of the myocardium (1.05g/mL).

In 40 patients, additional co-axial short-axis cine slices were acquired from the level of the aortic valve. The $LVOT_{area}$ was planimetered at the base of the aortic valve (the slice which all three cusps were first observed to disappear) in mid-systole and comparisons were made with the $LVOT_{area}$ estimated from the left ventricular outflow tract diameter on two-dimensional echocardiography (**Figure 3.1C**).

3.3.4 Curve Fitting and Statistical Analysis

In patients with normal stroke volumes, the relationship between aortic valve area and mean pressure gradient was modeled according to the Gorlin equation, aortic valve area = $c/\sqrt{\text{mean pressure gradient}}$ (GraphPad Prism 5, GraphPad Software Inc., San Diego, CA, USA). No rules were set for the initial value for the modeling parameter, *c*. We generated two curve-fitting models with aortic valve area derived using Doppler stroke volume and cardiovascular magnetic resonance stroke volume. Comparison between echocardiographic and cardiovascular magnetic resonance indices of stroke volume, $LVOT_{area}$ and aortic valve area was assessed using the Bland-Altman analyses. Fixed and proportional biases with 95% limits of agreement were reported. Standard statistical methods as described in Chapter 2 were also used and a two-sided $P<0.05$ was considered statistically significant.

3.4 RESULTS

A total of 133 patients with mild to severe aortic stenosis (aortic valve area 0.98±0.40 cm^2; mean pressure gradient 33±20 mmHg; peak aortic velocity 3.8±0.9 m/s) and 33 control individuals were recruited. The median interval between echocardiography and cardiovascular magnetic resonance was 9 [interquartile range: 5 to 29] days. Compared to control individuals, patients with aortic stenosis had higher ejection fractions (64±4 and 67±7%, respectively; P=0.02) despite similar left ventricular end-diastolic volumes (75±13 and 72±16 mL/m^2, respectively; P=0.34) and stroke volumes (47±8 and 48±10 mL/m^2, respectively; P=0.59) (**Table 3.1 and Table 3.2**).

In this study, 40 patients with mild to severe aortic stenosis were randomly selected and planimetry of the $LVOT_{area}$ was performed on cardiovascular magnetic resonance to investigate the effects of accurate $LVOT_{area}$ measurement on stroke volume estimation. The characteristics of these 40 patients were similar to the entire cohort of patients with aortic stenosis (**Table 3.3**).

TABLE 3.1. BASELINE CHARACTERISTICS OF PATIENTS WITH AORTIC STENOSIS AND CONTROL INDIVIDUALS

	Control Individuals (n=33)	Aortic Stenosis (n=133)	P value
Clinical Characteristics			
Age, years	54±23	68±12	<0.01
Males, n (%)	18 (55)	89 (67)	0.40
Hypertension, n (%)	9 (27)	85 (64)	<0.01
Diabetes Mellitus, n (%)	0	18 (14)	-
Coronary artery disease, n (%)	3 (9)	44 (33)	0.01
Atrial fibrillation, n (%)	0	3 (2)	-
Echocardiography			
Left ventricular outflow tract (LVOT) diameter, cm	2.05±0.17	2.07±0.24	0.66
LVOT cross-sectional area, cm^2	3.30±0.55	3.39±0.85	0.60
LVOT velocity time integral, cm	20.9±3.7	23.5±4.4	0.01
Doppler stroke volume, mL	70±19	79±19	<0.01
Doppler stroke volume (indexed), mL/m^2	38±8	42±10	<0.01
Aortic valve area, cm^2	2.36±0.59	0.98±0.40	<0.01
Aortic valve area (indexed), cm^2/m^2	1.26±0.26	0.52±0.21	<0.01
Mean pressure gradient, mmHg	4±1	33±20	<0.01
Peak aortic velocity, m/s	1.4±0.2	3.8±0.9	<0.01
Dimensionless index	0.72±0.10	0.28±0.09	<0.01
Aortic valve calcium score	1 [1,1]	3 [3,4]	<0.01
Valvuloarterial impedance, $mmHg \bullet ml^{-1} \bullet m^{-2}$	3.2±0.7	4.0±1.0	0.34
End-diastolic volume, mL¶	93±25	87±26	0.17
End-diastolic volume (indexed), mL/m^2¶	50±13	46±13	0.12
End-systolic volume, mL¶	41±14	38±14	0.29
End-systolic volume (indexed), mL/m^2¶	22±7	20±7	0.16
Stroke volume, mL¶	51±16	49±14	0.48
Stroke volume (indexed), mL/m^2¶	28±8	26±7	0.16
Ejection fraction, %¶	56±9	57±7	0.49
Mild mitral regurgitation, n (%)	2 (6)	19 (14)	0.37
Mild aortic regurgitation, n (%)	2 (6)	57 (43)	<0.01

	Control Individuals (n=33)	Aortic Stenosis (n=133)	P value
Cardiovascular Magnetic Resonance			
End-diastolic volume, mL	140±32	135±35	0.47
End-diastolic volume (indexed) (EDVi), mL/m^2	75±13	72±16	0.34
End-systolic volume, mL	51±15	46±18	0.14
End-systolic volume (indexed), mL/m^2	27±7	24±9	0.08
Stroke volume, mL	89±19	90±22	0.81
Stroke volume (indexed), mL/m^2	47±8	48±10	0.59
Ejection fraction, %	64±4	67±7	0.02
Left ventricular mass (indexed) (LVMi), g/m^2	67±15	89±22	<0.01
LVMi/EDVi, g/mL	0.90±0.13	1.25±0.26	<0.01

¶ Estimated using the Teichholz formula

TABLE 3.2. CHARACTERISTICS OF PATIENTS WITH AORTIC STENOSIS CLASSIFIED BASED ON AORTIC VALVE AREA ESTIMATED USING DOPPLER-DERIVED STROKE VOLUME [n]

	Non-severe (n=44)	Small-area low-gradient (n=56)	Severe (n=28)	P value
CLINICAL CHARACTERISTICS				
Age, years	65±13	72±10	68±11	0.02[a]
Males, n (%)	32 (72)	34 (61)	19 (68)	0.44
Height, cm	169±9	163±8	168±8	<0.01[a,b]
Body mass index, kg/m^2	29±5	29±5	27±4	0.13
Body surface area, m^2	1.9±0.2	1.8±0.2	1.9±0.2	0.07
Hypertension, n (%)	27 (61)	40 (71)	16 (57)	0.36
Diabetes Mellitus, n (%)	9 (20)	6 (11)	3 (11)	0.32
Coronary artery disease, n (%)	14 (32)	15 (27)	12 (43)	0.33
Atrial fibrillation, n (%)	0	3 (5)	0	-
Systolic blood pressure, mmHg	147±20	154±20	147±22	0.19
ECHOCARDIOGRAPHY				
Left ventricular outflow tract (LVOT) diameter, cm	2.19±0.21	1.96±0.19	2.08±0.24	<0.01[a,b]
LVOT cross-sectional area, cm^2	3.79±0.75	3.05±0.57	3.43±0.78	<0.01[a]
LVOT velocity time integral, cm	24.5±4.2	23.0±4.5	22.7±4.3	0.15
Doppler stroke volume, mL	92±18	70±13	78±19	<0.01[a,c]
Doppler stroke volume (indexed), mL/m^2	48±10	38±7	42±10	<0.01[a,c]
Aortic valve area, cm^2	1.38±0.38	0.79±0.15	0.69±0.17	<0.01[a,c]
Aortic valve area (indexed), cm^2/m^2	0.72±0.20	0.43±0.08	0.37±0.09	<0.01[a,c]
Mean pressure gradient, mmHg	20±8	29±9	54±17	<0.01[a,b,c]
Peak aortic velocity, m/s	3.0±0.5	3.7±0.5	4.8±0.6	<0.01[a,b,c]
Dimensionless index	0.36±0.09	0.26±0.05	0.20±0.04	<0.01[a,b,c]
Aortic valve calcium score	3 [2,3]	3 [3,4]	4 [4,4]	<0.01[a,b,c]
Valvuloarterial impedance, mmHg•ml^{-1}•m^{-2}	3.6±0.8	4.9±1.1	5.0±1.2	<0.01[a,c]

	Non-severe (n=44)	Small-area low-gradient (n=56)	Severe (n=28)	P value
ECHOCARDIOGRAPHY (CONTINUED)				
End-diastolic volume, mL[¶]	94±21	82±26	83±23	0.03[a]
End-diastolic volume (indexed), mL/m^2[¶]	49±10	45±13	44±12	0.15
End-systolic volume, mL[¶]	42±12	36±14	35±14	0.04
End-systolic volume (indexed), mL/m^2[¶]	22±6	19±7	19±7	0.11
Stroke volume, mL[¶]	53±12	46±13	48±13	0.04[a]
Stroke volume (indexed), mL/m^2[¶]	28±6	25±7	26±7	0.25
Ejection fraction, %[¶]	56±7	57±7	59±8	0.40
Mild mitral regurgitation, n (%)	3 (7)	9 (16)	7 (25)	0.10
Mild aortic regurgitation, n (%)	18 (41)	27 (48)	12 (43)	0.24
CARDIOVASCULAR MAGNETIC RESONANCE				
LVOT cross-sectional area, cm^2[‡]	4.22±1.21 (n=13)	3.58±0.83 (n=14)	4.53±1.24 (n=12)	0.09
End-diastolic volume, mL	142±30	126±25	139±40	0.03[a]
End-diastolic volume (indexed) (EDVi), mL/m^2	74±13	69±13	74±19	0.17
End-systolic volume, mL	47±17	43±15	47±20	0.38
End-systolic volume (indexed), mL/m^2	25±8	23±8	25±10	0.75
Stroke volume, mL	95±19	83±16	92±26	<0.01[a]
Stroke volume (indexed), mL/m^2	49±8	45±8	49±12	0.08
Ejection fraction, %	67±7	66±7	67±7	0.84
Left ventricular mass (indexed) (LVMi), g/m^2	85±18	85±21	99±25	<0.01[b,c]
LVMi/EDVi, g/mL	1.17±0.23	1.24±0.24	1.38±0.28	<0.01[b,c]

[π]5 patients were classified with large-area high-gradient aortic stenosis
[¶]Estimated using the Teichholz formula
[‡] Planimetered left ventricular outflow tract area was performed in 40 patients. One patient was classified with large-area high-gradient aortic stenosis

[a] P<0.05 between non-severe and small-area low-gradient aortic stenosis
[b] P<0.05 between small-area low-gradient and severe aortic stenosis
[c] P<0.05 between non-severe and severe aortic stenosis

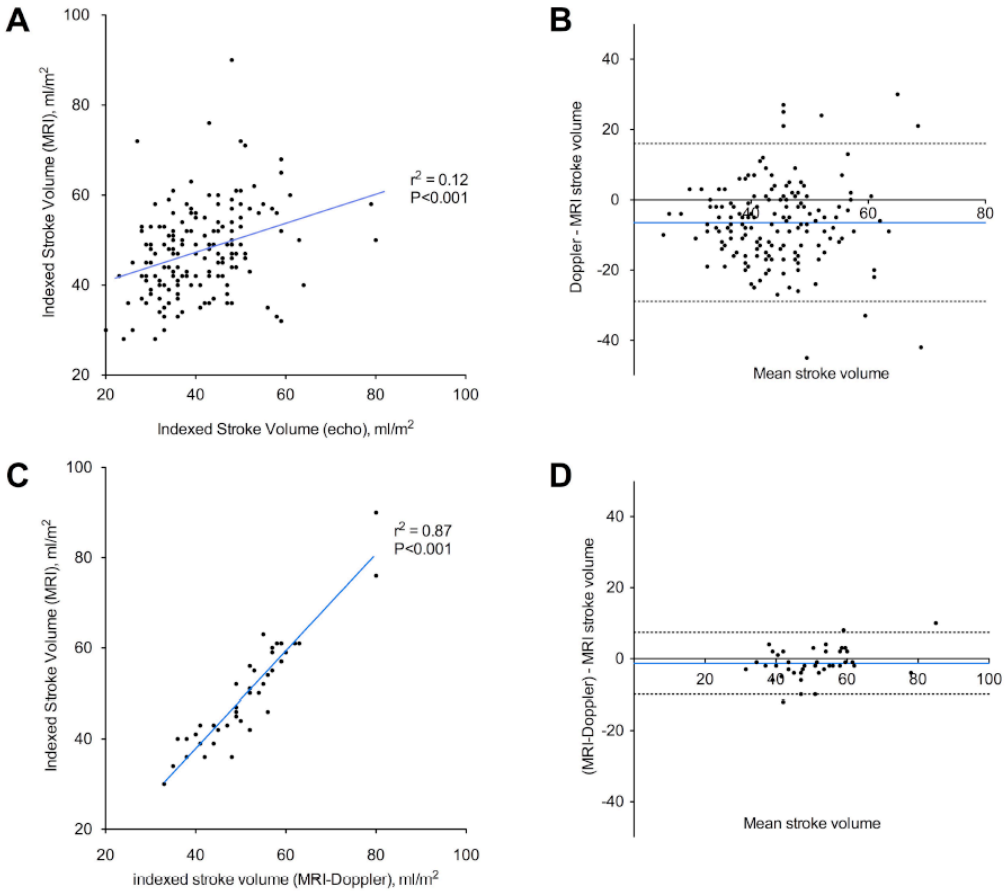
TABLE 3.3. BASELINE CHARACTERISTICS OF THE 40 PATIENTS WITH PLANIMETERED LEFT VENTRICULAR OUTFLOW TRACT AREA ON CARDIOVASCULAR MAGNETIC RESONANCE

Characteristics	Subgroup (n=40)	All patients with aortic stenosis (n=133)	P value
Age, years	68±12	69±12	0.64
Males, n (%)	27 (68)	83 (63)	0.56
Body surface index, m^2	1.9±0.2	1.9±0.2	1.00
Systolic blood pressure, mmHg	151±21	150±21	0.79
Heart rate, per min	64±10	64±11	1.00
Mean pressure gradient, mmHg	37±24	32±16	0.13
Peak aortic jet velocity, m/s	4.0±1.1	3.7±0.8	0.06
Aortic valve area, cm^2	1.0±0.3	1.0±0.4	1.00
End-diastolic volume (indexed) (EDVi), mL/m^2	75±21	72±16	0.34
Indexed end-systolic volume, mL/m^2	25±12	24±9	0.57
Indexed stroke volume, mL/m^2	50±12	48±10	0.29
Ejection fraction, %	67±8	67±7	1.00
Left ventricular mass (indexed) (LVMi), g/m^2	95±28	88±21	0.09
LVMi/EDVi, g/mL	1.29±0.28	1.25±0.26	0.40

3.4.1 Doppler and Cardiovascular Magnetic Resonance Stroke Volume

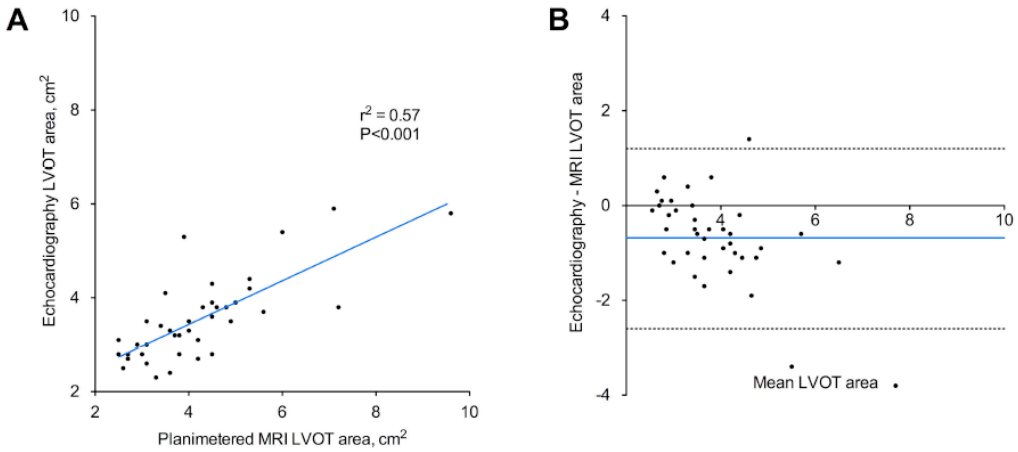
Doppler stroke volume correlated only weakly with cardiovascular magnetic resonance stroke volume measurements (r^2=0.12, P<0.001; **Figure 3.2A**) and underestimated the stroke volume by more than 6 mL/m^2 compared to cardiovascular magnetic resonance (-6.5 mL/m^2, 95% confidence interval: -28.9 to 16.0 mL/m^2; **Figure 3.2B**). Similar results were observed after excluding the 19 patients in the cohort with mild mitral regurgitation (r^2=0.14, P<0.001; mean difference -6.1 mL/m^2, 95% confidence interval: -28.2 to 16.0 mL/m^2). This in part appears to be due to underestimation of the $LVOT_{area}$ by echocardiography when compared with planimetered $LVOT_{area}$ measurements (-0.7 cm^2, 95% confidence interval: -2.6 to 1.3 cm^2; **Figure 3.3**). Indeed, when we subsequently recalculated stroke volume using the planimetered $LVOT_{area}$, an excellent correlation with cardiovascular magnetic resonance stroke volumes was observed (r^2=0.87, P<0.001; **Figure 3.2C**) without significant fixed or proportional biases (-1.3 mL/m^2, 95% confidence interval: -9.9 to 7.3 mL/m^2; **Figure 3.2D**). Moreover, this effect translated into an underestimation of the aortic valve area when calculated using echocardiography-derived stroke volumes compared with cardiovascular magnetic resonance measured stroke volumes (-0.23 cm^2, 95% confidence interval: -1.01 to 0.59 cm^2; **Figure 3.4**). As previously described the explanation for echocardiographic underestimation of the $LVOT_{area}$ appears related to its elliptic shape. Indeed, the mean ellipticity ratio (ratio of the maximum to minimum left ventricular outflow tract diameter) was 1.2±0.1, with only 28% of these patients having a circular left ventricular outflow tract (defined as ellipticity ratio of 1.0). Of note, we achieved excellent intra-observer (r^2=1.00, P<0.001; mean difference 0.5±2.7%) and inter-observer (r^2=0.98, P<0.001; mean difference 1.1±5.4%) agreement in the planimetered left ventricular outflow tract measurements using cardiovascular magnetic resonance.

FIGURE 3.2. STROKE VOLUME CORRELATION AND BLAND-ALTMAN ANALYSIS



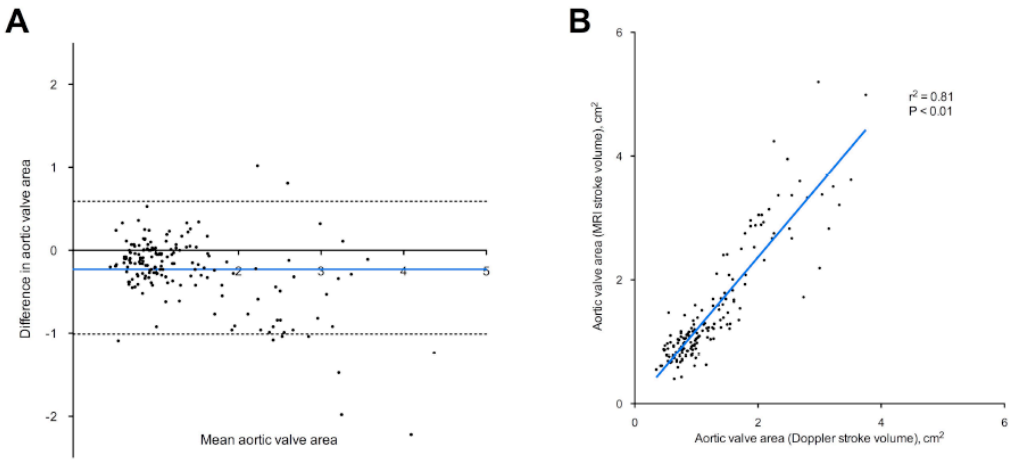
Doppler stroke volume correlated weakly with magnetic resonance imaging (MRI) stroke volume (**A**), with a fixed bias and wide limits of agreement (**B**). In 40 patients, stroke volume was calculated using planimetered left ventricular outflow tract area on MRI and Doppler left ventricular outflow tract flow (MRI-Doppler). This approach demonstrated excellent correlation with MRI stroke volume (**C**), without significant bias (**D**).

FIGURE 3.3. LEFT VENTRICULAR OUTFLOW TRACT (LVOT) AREA CORRELATION AND BLAND-ALTMAN ANALYSIS



Although left ventricular outflow tract area (LVOT) area estimated by echocardiography demonstrated a moderate correlation with planimetered LVOT area on magnetic resonance imaging (**A**), the echocardiographic LVOT area underestimated planimetered area with wide limits of agreement (**B**).

FIGURE 3.4. AORTIC VALVE AREA CORRELATION AND BLAND-ALTMAN ANALYSIS

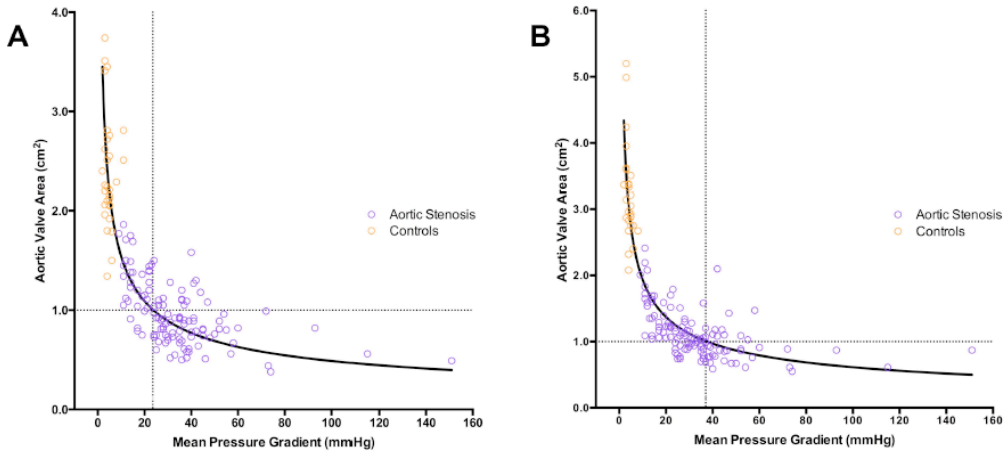


Aortic valve area estimated using Doppler stroke volume and MRI-derived stroke volume demonstrated poor agreement and significant underestimation (**A**), despite excellent correlation (**B**).

3.4.2 Consistent Aortic Valve Area and Mean Pressure Gradient Cutoffs

Based on measurements derived from Doppler stroke volume, a mean pressure gradient of 40 mmHg corresponded to an aortic valve area of 0.77 cm^2, whilst an aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of only 24 mmHg (aortic valve area = 4.85/√mean pressure gradient; r^2=0.73; **Figure 3.5A**). When cardiovascular magnetic resonance stroke volume measurements were used to calculate the aortic valve area, a mean pressure gradient of 40 mmHg corresponded to an aortic valve area of 0.97 cm^2 and an aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of 37 mmHg (aortic valve area = 6.13/√mean pressure gradient; r^2=0.81, **Figure 3.5B**).

FIGURE 3.5. RELATIONSHIP BETWEEN AORTIC VALVE AREA AND MEAN PRESSURE GRADIENT



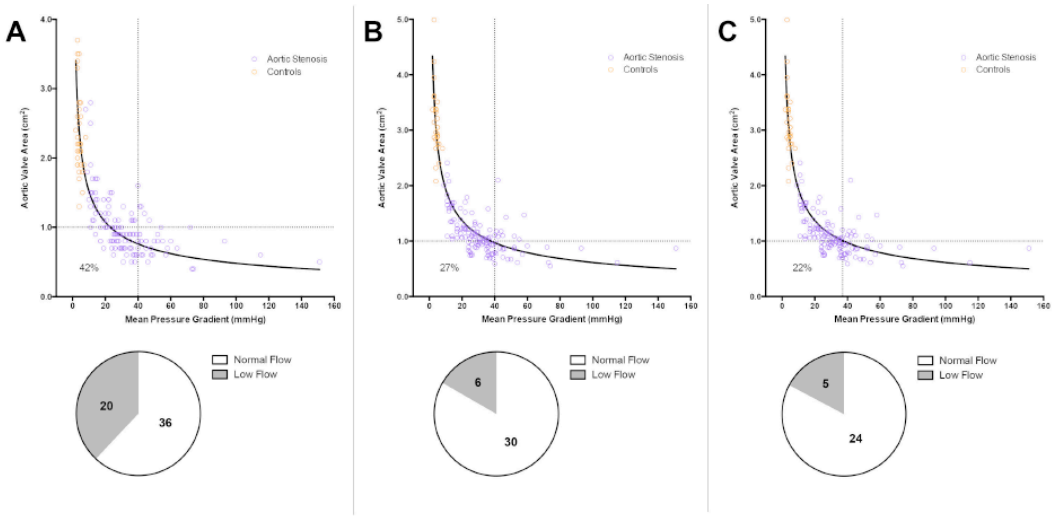
The aortic valve area was calculated from the continuity equation using Doppler stroke volume. An aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of 24 mmHg (**A**). Correcting these values using the magnetic resonance imaging stroke volume, an aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of 37 mmHg (**B**).

3.4.3 Discordant Small-area Low-gradient Aortic Stenosis

Using the conventional echocardiographic estimation of mean pressure gradient and aortic valve area, and the thresholds for severe disease based on current guidelines (aortic valve area of 1.0 cm^2 and mean pressure gradient of 40 mmHg) (141),(5), 56 patients with aortic stenosis (42%) had discordant small-area low-gradient aortic stenosis (**Figure 3.6A**).

Using a step-wise approach, we first assessed the impact of using aortic valve area measurements derived from cardiovascular magnetic resonance stroke volumes on this proportion of patients with discordant small-area low-gradient aortic stenosis. This resulted in 20 patients being reclassified as having non-severe aortic stenosis (median aortic valve calcium score 3; valvuloarterial impedance 3.7±0.7 $mmHg/mL/m^2$), leaving 36 with small-area low-gradient aortic stenosis (**Figure 3.6B**). Subsequently when we used the revised thresholds established above (aortic valve area of 1.0 cm^2 and mean pressure gradient of 37 mmHg), a further 7 patients were reclassified with severe disease (all had aortic valve calcium score of 4 and valvuloarterial impedance 4.5±1.2 $mmHg/mL/m^2$). This left only 29 patients with discordant small-area low-gradient aortic stenosis, a reduction of 48% compared to the original classification (**Figure 3.6C**). Of these, three patients had impaired systolic function and two had a low stroke volume due to small left ventricular cavity volumes. The remainder appeared to consist of patients with moderate to severe disease with values for a wide range of parameters that were intermediate between concordant moderate and severe disease (**Table 3.4**). This included the aortic valve calcium score, which was 3 in 48% and 4 in 52% of patients.

FIGURE 3.6. RECLASSIFICATION OF AORTIC STENOSIS SEVERITY



Using traditional echocardiographic measurements and the recommended severity cutoffs established in current guidelines (**A**), 56 patients had discordant small-area low-gradient aortic stenosis (AS). Twenty patients were reclassified to concordant non-severe AS when cardiovascular magnetic resonance stroke volume was used to estimate aortic valve area (**B**). A further seven patients were reclassified as having concordant severe disease using the revised thresholds of 1.0 cm^2 and 37 mmHg (**C**). The corresponding pie charts show the flow states in patients with discordant small-area low-gradient aortic stenosis (stroke volume estimated using cardiovascular magnetic resonance).

TABLE 3.4. CHARACTERISTICS OF PATIENTS WITH DISCORDANT SMALL-AREA LOW-GRADIENT AORTIC STENOSIS AFTER CORRECTION FOR STROKE VOLUME UNDERESTIMATION AND INCONSISTENT THRESHOLDS [π]

	Non-severe (n=61)	Small-area low-gradient (n=29)	Severe (n=33)	P value
CLINICAL CHARACTERISTICS				
Age, years	66±13	73±9	72±9	<0.01[a,c]
Male, n (%)	44 (72)	15 (52)	20 (61)	0.15
Height, cm	168±9	161±9	165±8	<0.01[a]
Body mass index, kg/m^2	29±5	30±5	27±3	0.09
Body surface area, m^2	1.9±0.2	1.8±0.2	1.8±0.2	0.02
Hypertension, n (%)	38 (62)	21 (72)	22 (67)	0.64
Diabetes mellitus, n (%)	11 (18)	3 (10)	4 (12)	0.56
Coronary artery disease, n (%)	18 (30)	7 (24)	17 (52)	0.04
Atrial fibrillation, n (%)	-	3 (10)	-	-
Systolic blood pressure, mmHg	149±21	151±22	151±23	0.91
ECHOCARDIOGRAPHY				
Left ventricular outflow tract (LVOT) diameter, cm	2.14±0.21	1.94±0.21	2.02±0.25	<0.01[a,c]
LVOT cross-sectional area, cm^2	3.64±0.73	3.01±0.63	3.28±0.82	<0.01[a]
LVOT velocity time integral, cm	23.6±4.2	23.3±5.1	23.7±4.4	0.93
Doppler stroke volume, mL	86±19	69±14	77±20	<0.01[a]
Doppler stroke volume (indexed), mL/m^2	45±10	38±8	42±10	<0.01[a]
Aortic valve area, cm^2	1.24±0.41	0.76±0.16	0.71±0.19	<0.01[a,c]
Aortic valve area (indexed), cm^2/m^2	0.65±0.22	0.42±0.10	0.39±0.10	<0.01[a,c]
Mean pressure gradient, mmHg	21±8	30±5	55±24	<0.01[a,b,c]
Peak aortic velocity, m/s	3.1±0.6	3.7±0.3	4.8±0.9	<0.01[a,b,c]
Dimensionless index	0.34±0.09	0.26±0.05	0.21±0.05	<0.01[a,b,c]
Aortic valve calcium score	3 [2,3]	4 [3,4]	4 [4,4]	<0.01[a,b,c]
Valvuloarterial impedance, mmHg•ml^{-1}•m^{-2}	4.0±1.0	4.8±1.2	4.9±1.3	<0.01[a,c]

	Non-severe (n=61)	Small-area low-gradient (n=29)	Severe (n=33)	P value
ECHOCARDIOGRAPHY (CONTINUED)				
End-diastolic volume, mL¶	93±25	77±25	83±24	<0.01[a]
End-diastolic volume (indexed), mL/m²¶	49±12	42±13	45±13	0.09
End-systolic volume, mL¶	42±14	31±13	35±14	<0.01[a]
End-systolic volume (indexed), mL/m²¶	22±7	17±7	20±7	0.02[a]
Stroke volume, mL ¶	52±13	45±13	45±11	0.02
Stroke volume (indexed), mL/m²¶	27±6	25±7	25±7	0.27
Ejection fraction, % ¶	56±6	60±7	57±9	0.05[a]
Mild mitral regurgitation, n (%)	7 (11)	4 (14)	7 (21)	0.44
Mild aortic regurgitation, n (%)	24 (39)	16 (56)	16 (48)	0.34
CARDIOVASCULAR MAGNETIC RESONANCE				
LVOT cross-sectional area, cm²‡	4.30±1.05 (n=16)	3.40±0.90 (n=8)	4.10±1.36 (n=12)	0.20
End-diastolic volume, mL	142±28	117±18	127±35	<0.01[a,c]
End-diastolic volume (indexed) (EDVi), mL/m²	74±14	65±9	70±17	0.01[a]
End-systolic volume, mL	47±16	39±10	45±22	0.12
End-systolic volume (indexed), mL/m²	25±8	22±5	25±11	0.28
Stroke volume, mL	95±18	78±13	83±21	<0.01[a,c]
Stroke volume (indexed), mL/m²	50±9	43±7	45±10	<0.01[a]
Ejection fraction, %	67±7	67±6	66±9	0.63
Left ventricular mass (indexed) (LVMi), g/m²	86±21	81±18	93±20	0.06
LVMi/EDVi, g/mL	1.18±0.22	1.25±0.23	1.37±0.30	<0.01[c]

[π]10 patients were classified with large-area high-gradient aortic stenosis
¶ Estimated using the Teichholz formula
‡ Planimetered left ventricular outflow tract area was performed in 40 patients. Four patients were classified with large-area high-gradient aortic stenosis

[a] P<0.05 between non-severe and small-area low-gradient aortic stenosis
[b] P<0.05 between small-area low-gradient and severe aortic stenosis
[c] P<0.05 between non-severe and severe aortic stenosis

3.4.4 Stroke Volume Estimation and Aortic Stenosis Classification Using the Teichholz Formula

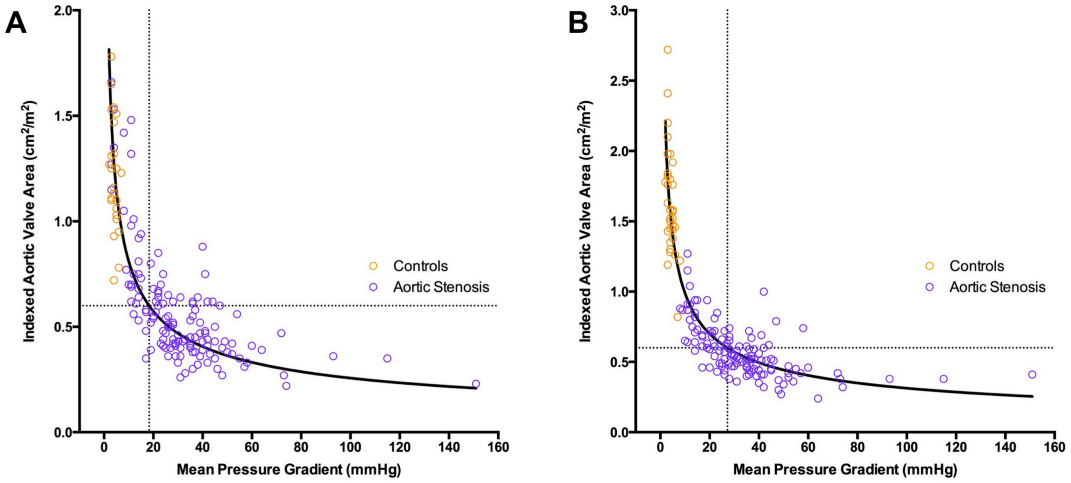
In a further analysis, we assessed an alternate echocardiographic method for estimating stroke volumes using the Teichholz formula (139). Results were similar with the correlation between echocardiography-estimated and cardiovascular magnetic resonance derived stroke volumes remaining weak (r^2=0.16, P<0.001), and 51% of patients classified as having discordant small-area low-gradient aortic stenosis.

3.4.5 Evaluation of Aortic Stenosis Classification Using Indexed Aortic Valve Area and the Dimensionless Index

We investigated thresholds of severe aortic stenosis using indexed aortic valve area, and the effects on classification using an indexed aortic valve area of 0.6 cm^2/m^2 and mean pressure gradient of 40 mmHg.

Using Doppler stroke volume, an indexed aortic valve area of 0.6 cm^2/m^2 corresponded to a mean pressure of 18 mmHg while an indexed valve area of 0.6 cm^2/m^2 corresponded to a mean pressure gradient of 27 mmHg with cardiovascular magnetic resonance derived stroke volume (**Figure 3.7**). The use of indexed aortic valve area did not reduce the number of patients with discordant small-area low-gradient aortic stenosis with either Doppler stroke volume [61 patients (46%) compared with the 56 patients (42%) using non-indexed aortic valve area] or cardiovascular magnetic resonance derived stroke volumes [52 patients (39%) compared with the 36 patients (27%) using non-indexed aortic valve area].

FIGURE 3.7. AORTIC STENOSIS CLASSIFICATION USING INDEXED AORTIC VALVE AREA



Using a dimensionless index (DI) threshold of <0.25 and mean pressure gradient of <40 mmHg, 26 patients were classified with discordant low-DI low-gradient aortic stenosis (20%). This appears to support out conclusion that discordant small-area low-gradient aortic stenosis is largely influenced by $LVOT_{area}$ estimation.

However, this result has to be interpreted with caution. The use of dimensionless index has limitations precisely because it does not take into account the $LVOT_{area}$, which is the key factor to consider when determining the severity of aortic stenosis (7,142). This is perhaps best illustrated with an example:

Aortic valve area = $LVOTd^2$ x 0.785 x DI; DI = $LVOT_{VTI}/AV_{VTI}$

In a patient with a LVOT diameter (LVOTd) of 2.0cm and DI of 0.25 (severe aortic stenosis), this would translate to an aortic valve area of 0.79 cm^2 (severe aortic stenosis). However, in another patient with LVOTd of 2.5cm and the same DI of 0.25, this increases the aortic valve area to 1.23 cm^2 (moderate aortic stenosis). This example illustrates that a DI threshold of 0.25 may not be appropriate in all patients: in patients with large left ventricular outflow tract, a smaller dimensionless index threshold for severe disease may be needed (142). Indeed, amongst the 26 patients with discordant low-DI low-gradient aortic stenosis, 9 patients (35%) had an aortic valve area > 1.0 cm^2 and they had a larger mean left ventricular outflow tract diameter (measured on echocardiography) compared to the other 17 patients (2.2±0.2 versus 1.9±0.2 cm; P=0.03).

3.4.6 Comparison of Doppler, MRI Volumetric and Phase Contrast Stroke Volume Estimation

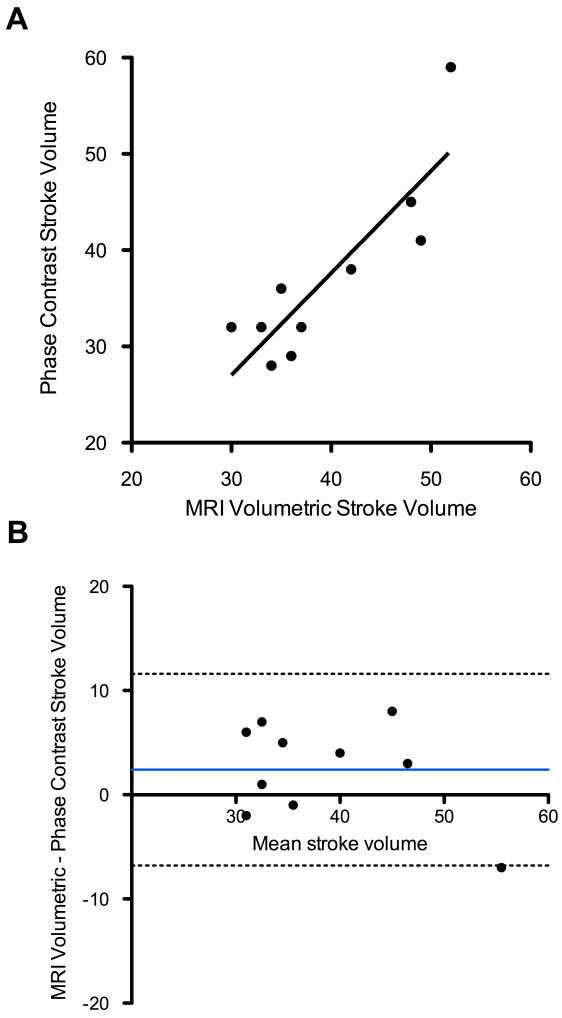
An exploratory analysis was performed in 10 patients with aortic stenosis to compare Doppler, MRI volumetric (cine) and phase contrast stroke volume. Through plane phase contrast velocity mapping was positioned orthogonal to the ascending aorta at the level of the bifurcation of the main pulmonary artery. An initial velocity encoding level of 100 cm/s was selected and increased in increments of 50 cm/s if aliasing occurs. The total forward flow during systole is computed using the Argus software (Siemens AG Healthcare Sector, Erlangen, Germany).

The results are shown in **Table 3.5**. In these 10 patients, there was no correlation between Doppler indexed stroke volume and MRI-derived indexed stroke volume (r=0.32; P=0.37) and between Doppler indexed stroke volume and MRI phase contrast indexed stroke volume (r=0.20; P=0.58). On the other hand, MRI-derived stroke volume and MRI phase contrast demonstrated excellent correlation (r=0.87; P=0.001; **Figure 3.8**) and agreement (2.4mL/m^2; 95% CI -6.8 to 11.6 mL/m^2; **Figure 3.8**).

TABLE 3.5. COMPARISON OF STROKE VOLUME ESTIMATION

Patient S/N	Echocardiographic indexed stroke volume (mL/m^2)	MRI-derived indexed stroke volume (mL/m^2)	Phase contrast indexed flow volume (mL/m^2)	Aortic stenosis severity
#1	34	49	41	Severe
#2	33	33	32	Severe
#3	45	34	28	Moderate
#4	35	35	36	Moderate
#5	46	37	32	Severe
#6	54	36	29	Severe
#7	50	48	45	Mild
#8	45	42	38	Moderate
#9	36	30	32	Severe
#10	49	52	59	Mild

FIGURE 3.8. CORRELATION AND BLAND-ALTMAN ANALYSIS BETWEEN MRI VOLUMETRIC AND PHASE CONTRAST STROKE VOLUME



Stroke volumes derived using MRI volumetric estimation and phase contrast demonstrated excellent correlation (**A**) and narrow limits of agreement (**B**).

3.5 DISCUSSION

In this study, we have systematically demonstrated that echocardiography underestimates the $LVOT_{area}$, the left ventricular stroke volume, and as a consequence, the aortic valve area. Moreover we have demonstrated that there are inconsistencies in the guideline thresholds of severity with an aortic valve area of 1.0 cm^2 corresponding to a mean pressure gradient of 24 mmHg based on standard echocardiographic measures and 37 mmHg when cardiovascular magnetic resonance derived stroke volumes are used. Finally we have shown that if we correct for these two factors using more accurate cardiovascular magnetic resonance estimation of the stroke volume to calculate aortic valve area and revised thresholds, more than 40% of the patients with small-area low-gradient aortic stenosis were reclassified as having concordant measurements.

Two-dimensional and Doppler echocardiography assessments are the predominant methods used worldwide to assess the severity of aortic stenosis. However, the echocardiographic estimation of the aortic valve area relies on accurate measurement of stroke volume. Unfortunately as demonstrated in this study, echocardiography frequently underestimates the stroke volume when compared to non-invasive gold-standard measurements made using cardiovascular magnetic resonance. As a consequence, echocardiography would also appear to underestimate the aortic valve area. Our data provide explanations for these observations. A subgroup of 40 patients had co-axial short-axis cine images of their left ventricular outflow tract. This allowed accurate and reproducible planimetered measurements of the $LVOT_{area}$ to be compared to the derived measurements made using two-dimensional echocardiographic diameter measurements. Similar to previous studies (136,137), we have demonstrated that such echocardiographic measures underestimate the true $LVOT_{area}$ in part due to the fact that the left ventricular outflow tract is frequently elliptical not circular. Indeed, when Doppler stroke volumes were corrected using the more accurate planimetered measurements of the $LVOT_{area}$, a good correlation with cardiovascular magnetic resonance derived stroke volumes

was subsequently observed. Further to our analyses, we have also explored using other echocardiographic indices such as indexed aortic valve area and the dimensionless index. Unfortunately, these techniques were also associated with inherent limitations related to the $LVOT_{area}$ measurements.

Inconsistencies in the MPG and AVA thresholds recommended by the current guidelines are well described (15,130,142). Consistent with previous reports (15,130), our echocardiography data confirmed an aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of only 24 mmHg, significantly lower than the threshold of 40 mmHg stated in current guidelines. Interestingly this improved to 37 mmHg when cardiovascular magnetic resonance stroke volume measurements were used to calculate aortic valve area, much closer to the recommended threshold though still discrepant.

Multiple previous studies have shown that a third of patients with moderate and severe aortic stenosis have discordant disease severity according to their aortic valve areas and mean pressure gradients. Interest has surrounded this group given its ubiquity and the uncertainty in the outcome associated with these patients. Indeed whilst some studies have suggested that patients with small-area low-gradient aortic stenosis have a prognosis similar to those with moderate disease, others have indicated the exact opposite and that their outcomes are more akin to those with severe disease (132-135).

In the final part of the study, we investigated whether the underestimation of the aortic valve area by echocardiography and inconsistencies in the guideline thresholds might explain the ubiquity of patients with small-area low-gradient aortic stenosis and help resolve the true severity of their disease. We demonstrated that correcting for these two factors reduced the number of patients with a small-area low-gradient by over 40%. Of the remaining 29 subjects, 3 had low flow due to an impaired ejection fraction and 2 had low flow due to small LV cavity size. The remainder appeared to genuinely sit on the borderline between moderate and severe disease with parameters that were intermediate between those observed in concordant

severe and non-severe groups. Our data would therefore indicate that discordance in the assessment of aortic stenosis severity can be reduced by correcting for aortic valve area underestimation and inconsistent thresholds, but further studies are now needed to investigate the long-term outcomes of patients reclassified using this approach.

3.5.1 Study Limitations

In this study, assessment of the planimetered $LVOT_{area}$ on cardiovascular magnetic resonance was only available in 40 patients. However, this was felt to be a large enough sample size to assess the inaccuracies associated with left ventricular outflow tract diameter measurements and the data is consistent with the large and expanding literature investigating $LVOT_{area}$ measurements for the sizing of transcatheter aortic valve bioprostheses (137). Moreover, the baseline characteristics were similar between these 40 patients and the entire cohort of patients with aortic stenosis. We also used echocardiography to assess aortic valve calcification. Whilst this provides important prognostic information (140), computed tomography gives a more sensitive quantification of aortic valve calcification and has recently been shown to provide differentiation as to the true severity of patients with small-area low-gradient aortic stenosis (131,143). Phase contrast cardiovascular magnetic resonance is an alternate method to estimate stroke volume, but this technique is associated with its own problems particularly in patients with aortic stenosis where complex aortic flow in the ascending aorta can result in measurement inaccuracy. This is a particular problem at 3T. Finally, we were not able to perform both echocardiography and cardiovascular magnetic resonance on the same day because many of our elderly patients could not tolerate both procedures on the same visit. However, no patient experienced any cardiac events or changes in medications between the two scans and after correcting for inaccuracies in the $LVOT_{area}$, an excellent agreement was observed between cardiovascular magnetic resonance and echocardiography-derived stroke volumes. This would argue against any significant variability in stroke volumes between the scans.

3.6 CONCLUSIONS

Echocardiography underestimates the aortic valve area because of an underestimation of the $LVOT_{area}$ and stroke volume, compared to cardiovascular magnetic resonance. These factors, along with inconsistent aortic valve area and mean pressure gradient cutoffs in the current guidelines, account for more than 40% of patients with discordant small-area low-gradient aortic stenosis.

CHAPTER 4

OPTIMIZATION AND COMPARISON OF MYOCARDIAL T1 TECHNIQUES AT 3T IN PATIENTS WITH AORTIC STENOSIS

Published in:

Chin CW, Semple S, Malley T, White AC, Mirsadraee S, Weale PJ, Prasad S, Newby DE, Dweck MR. Optimization and comparison of myocardial T1 techniques at 3T in patients with aortic stenosis. **Eur Heart J Cardiovasc Imaging.** 2014;15(5):56-565.

4.1 SUMMARY

AIMS: To determine the optimal T1 mapping approach to assess myocardial fibrosis at 3T

METHODS: T1 mapping was performed at 3T using the modified look-locker-inversion sequence in 20 healthy volunteers and 20 patients with aortic stenosis. Pre- and post-contrast myocardial T1, the partition coefficient (λ; $\Delta R_{myocardium}/\Delta R_{blood}$, where $\Delta R = 1/\text{post-contrast T1} - 1/\text{pre-contrast T1}$) and extracellular volume fraction (ECV; λ x [1-haematocrit]) were assessed. After establishing the optimal time-point and myocardial region for analysis, we compared the reproducibility of these T1 measures and their ability to differentiate asymptomatic patients with aortic stenosis from healthy volunteers.

RESULTS: There was no segmental variation across the ventricle in any of the T1 measures evaluated. λ and ECV did not vary with time, while post-contrast T1 was relatively constant between 15-30min. Thus, mid-cavity myocardium at 20min was used for subsequent analyses. ECV displayed excellent intra-, inter-observer, and scan-rescan reproducibility (intraclass correlation coefficients (ICC) 1.00, 0.97 and 0.96 respectively), as did λ (ICC 0.99, 0.94, 0.93 respectively). Moreover, ECV and λ were both higher in patients with aortic stenosis compared to controls (ECV 28.3±1.7 *versus* 26.0±1.6%, P<0.001; λ 0.46±0.03 *versus* 0.44±0.03, P=0.02), with the former offering improved differentiation. By comparison, scan-rescan reproducibilities for pre- and post-contrast myocardial T1 were only modest (ICC 0.72 and 0.56) with no differences in values observed between cases and controls (both P>0.05).

CONCLUSIONS: ECV appears to be the most promising measure of diffuse myocardial fibrosis at 3T based upon their superior reproducibility and ability to differentiate disease from health.

4.2 INTRODUCTION

Myocardial fibrosis is a common pathological finding in a wide range of cardiovascular diseases and has been associated with an adverse prognosis (58,144,145). Using cardiovascular magnetic resonance (CMR), the late gadolinium enhancement technique has become widely used to evaluate focal myocardial fibrosis. However in many conditions, including aortic stenosis, a more diffuse form of fibrosis predominates, which crucially is reversible and therefore a potential target for novel therapeutic strategies (36,47,96,146). Late gadolinium enhancement imaging has inherent limitations in assessing diffuse fibrosis because it relies upon detecting a difference in signal intensity between normal and fibrotic regions (44). Consequently, it has difficulty in discriminating areas of diffuse myocardial fibrosis, which tend to have an even distribution.

Recently, several T1 mapping approaches have been developed to quantify diffuse fibrosis. The first approach measures intrinsic myocardial T1 on the basis that T1 relaxation times are longer in regions of fibrosis (pre-contrast T1) (110,147,148). Alternatively, myocardial T1 can be measured following gadolinium administration, which accumulates in fibrotic areas on account of the increased extracellular volume (post-contrast T1) (149,150). However, post-contrast T1 is potentially confounded by individual variations in gadolinium kinetics and by the precise timing of imaging (44). As a result, investigators have proposed methods to correct for these factors using either blood-pool T1 values to derive the partition coefficient (151), or plasma volume to calculate the contrast volume of distribution in the myocardium. The latter is commonly referred to as the myocardial extracellular volume fraction (152-157). Each of these approaches have been validated against the extent of myocardial fibrosis on histology (149,150,152,154,155,157). However, the optimal technique remains uncertain due to a lack of consistent acquisition sequences and disease states studied, whilst direct comparative studies are relatively lacking (157). In addition, there is insufficient reproducibility data (particularly scan-rescan) and few studies have been

performed at 3T (158-161), which may offer potential improvements compared to 1.5T (162).

Therefore, the purpose of this study was to perform a systematic and comprehensive assessment to determine the optimal T1 approach at 3T. In particular, we aimed to characterize the temporal and regional T1 profiles of the myocardium; and to identify the optimal technique based upon its reproducibility and ability to differentiate asymptomatic patients with aortic stenosis from healthy volunteers. Patients with advanced symptoms and focal scarring were excluded so as to focus on patients in whom diffuse myocardial fibrosis is most likely to be of clinical interest.

4.3 METHODS

4.3.1 Study Population

Twenty asymptomatic patients with mild to severe aortic stenosis were recruited from outpatient clinics at the Edinburgh Heart Centre. Twenty healthy volunteers were recruited from the community and the University of Edinburgh. All individuals had normal renal function and a left ventricular ejection fraction within the normal range. In addition to the exclusion criteria stated in Chapter 2, patients with aortic stenosis and presence of focal late gadolinium enhancement were excluded in this study. Exclusion criteria for healthy volunteers were: (1) hypertension, (2) diabetes mellitus, (3) coronary artery disease (previous myocardial infarction, evidence of myocardial ischemia, or >50% luminal stenosis in a major epicardial vessel) (4) valvular heart disease, (5) cardiomyopathy or previous myocarditis and (6) the presence of focal late gadolinium enhancement.

All clinical assessments and imaging studies were carried out at the Wellcome Trust Clinical Research Facility and the Clinical Research Imaging Centre, Edinburgh. Studies were performed with the approval of the local research ethics committee, and with the written informed consent from each participant.

4.3.2 Imaging Protocols and Analysis

The echocardiographic and cardiovascular magnetic resonance imaging protocols for assessing aortic stenosis severity, cardiac function and myocardial morphology (including myocardial fibrosis assessment with late gadolinium enhancement and novel myocardial T1 mapping) have been described in detail in Chapter 2. Furthermore, cardiovascular magnetic resonance imaging was performed at additional time-points to assess temporal changes in T1 measures (**Figure 4.1**).

Myocardial T1 values were assessed at multiple time-points to establish the optimal time-point for post-contrast T1 mapping. This was defined at the flattest point on the T1 relaxation curve at which variation in T1 values with time was at a minimum. Mid-cavity motion-corrected T1 maps were analyzed using a dedicated workstation (OsiriX version 4.1.1, Geneva, Switzerland). To minimize partial volume effects from surrounding tissues and blood-pool, we standardized the windowing and placement of regions of interest around the mid-cavity myocardium using a pre-defined protocol (**Figure 4.2**). The regions of interest were first drawn on the pre-contrast T1 maps and then copied onto each of the corresponding post-contrast T1 maps with stringent adjustments applied to avoid blood-pool and artifact (**Figure 4.2A**). This approach ensured consistency in the placement of regions of interest across the different time points, allowing us to investigate the temporal variation in our T1 measures.

Myocardial partition coefficient (λ) and extracellular volume fractions (ECV) were also calculated at all time-points. These measures were derived from pre- and post-contrast myocardial T1 values corrected for blood-pool T1 (measured at the mid-cavity, **Figure 4.2**) and haematocrit (sampled at the time of CMR), according to:

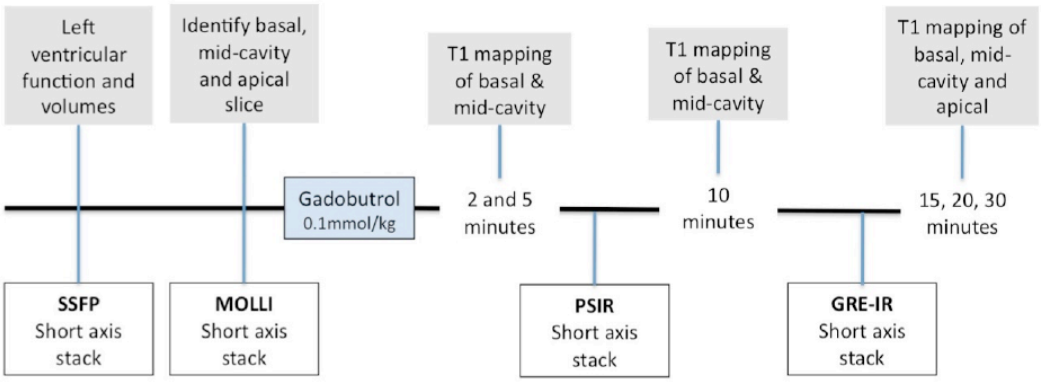
$$\lambda = \Delta R1_{myocardium} / \Delta R1_{blood\text{-}pool}, \text{ where } R1=1/T1 \quad [1]$$

$$ECV = (1\text{-haematocrit}) \times \lambda \quad [2]$$

Using a standardized approach, the basal, mid-cavity and apical T1 maps were divided into segments according to the standard 17-segment model recommended by the American College of Cardiology/American Heart Association (163). We excluded the true apex because it was not possible to avoid partial volume effects. Regions of interest were drawn in each of the 16 segments on the pre-contrast T1 map and subsequently, copied onto the post-contrast T1 map at the optimal time-point established from the above analysis (**Figure 4.2B**). Pre- and post-contrast myocardial T1, partition coefficient and extracellular volume fraction values were assessed in each segment of the left ventricle.

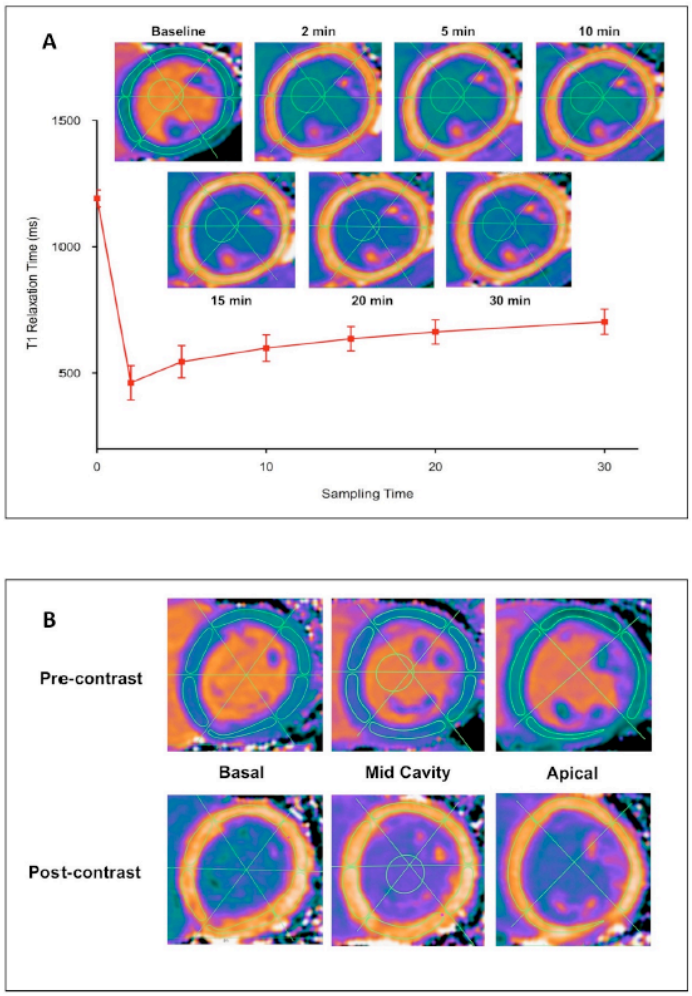
We established the reproducibility of our image analysis technique and of the different T1 measures from a random sample of 5 healthy volunteers and 5 patients with aortic stenosis. In assessing intra-observer reproducibility, a single observer repeated analysis after an interval of more than two weeks to minimize recall bias. For inter-observer reproducibility, two independent observers performed separate blinded analyses. Scan-rescan reproducibility was assessed in 10 healthy volunteers. Repeat scans were performed at least 7 days after the first scan, and haematocrit samples were collected on both scan days. Images were analyzed as above by a single observer.

FIGURE 4.1.CARDIOVASCULAR MAGNETIC RESONANCE IMAGING AND MYOCARDIAL T1 MAPPING PROTOCOL



Cardiovascular magnetic resonance imaging was performed in all patients with aortic stenosis and healthy volunteers (n=40) according to the study protocol

FIGURE 4.2. METHODOLOGY FOR MEASURING MYOCARDIAL T1 AT MULTIPLE TIME-POINTS AND IN MULTIPLE SEGMENTS OF THE LEFT VENTRICLE



(A) Measurement of myocardial T1 at multiple time points. Regions of interest were drawn within the borders on the pre-contrast myocardial T1 maps and then copied onto the corresponding post-contrast images at all time points. Minor adjustments were made to avoid artifacts and blood pool. A region of interest was also drawn in the left ventricular blood pool in order to calculate the partition coefficient and extracellular volume fraction at each time point. **(B)** Assessment of regional variation in T1 measures. Using the anterior and inferior ventricular insertion points as well as the mid-point of the ventricular cavity as reference points, three intersecting lines were drawn to divide the left ventricle into 16 segments. Regions of interest were drawn onto the basal (6 segments), mid-cavity (6 segments) and apical (4 segments) pre-contrast T1 maps with the standardized approach described above. Subsequently, the regions of interest were copied onto the 20-min post-contrast T1 maps. Pre- and post-contrast T1, partition coefficients and extracellular volume fraction values were assessed in each segment.

4.3.3 Statistical Analysis

Sample size estimation was not performed because this is an exploratory study to optimize a novel technique and determine the T1-derived measure with the most potential to assess diffuse myocardial fibrosis.

In all T1-derived measures, we compared the values across all the segments using one-way analysis of variance (ANOVA) with *post-hoc* Bonferroni adjustment. We examined the potential influence of heart rate and age on T1 measures using univariate linear analysis, and adjusted the effects of age and haematocrit using multivariate linear regression. Reproducibility analysis (intra-, inter-observer, and scan-rescan) was performed using intraclass correlation coefficients (ICC). ICC values between 0.50 and 0.75 indicated moderate reliability, and values >0.75 good reliability. For clinical measures, excellent intraclass correlation coefficients of >0.90 are required to ensure sufficient reliability (164). Fixed and proportional biases with 95% limits of agreement were assessed using Bland-Altman analyses.

The standard statistical analyses described in Chapter 2 and Bland-Altman analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). Univariate and multivariate linear regression, and ICC were performed using SPSS version 19 (SPSS, Inc., Chicago, IL, USA). A two-sided $P<0.05$ was considered statistically significant.

4.4 RESULTS

Patients with aortic stenosis were older than healthy volunteers (median age 75 *versus* 55 years, P<0.01) and there were an equal number of males and females (**Table 4.1**). On average they had moderate aortic stenosis (mean aortic valve area 1.2±0.6 cm^2; peak aortic valve velocity 3.3±0.9 m/s) with an increased left ventricular mass index and indices of diastolic dysfunction compared to healthy volunteers (**Table 4.1**).

A total of 1215 myocardial segments were analyzed (606 in healthy volunteers and 609 in patients with aortic stenosis) and 5.1% of segments were rejected because of artifacts.

TABLE 4.1. BASELINE CLINICAL, ECHOCARDIOGRAPHIC AND CARDIOVASCULAR MAGNETIC RESONANCE CHARACTERISTICS

	Healthy Volunteers	Aortic Stenosis	P value
CLINICAL CHARACTERISTICS			
Males, n (%)	10 (50)	10 (50)	1.00
Median age, years [IQR]	55 [22,65]	71 [53,75]	<0.01
Hypertension, n (%)	0	12 (60)	-
Diabetes Mellitus, n (%)	0	3 (15)	-
Coronary artery disease, n (%)	0	4 (20)	-
Systolic blood pressure, mmHg	140±12	153±25	0.05
Heart rate, beats/min	67±11	62±10	0.14
Haematocrit	0.41±0.03	0.39±0.04	0.05
MEDICATIONS			
Aspirin, n (%)	0	6 (30)	-
ACEI/ARB, n (%)	0	6 (30)	-
Beta blockers, n (%)	0	3 (15)	-
Statin therapy, n (%)	0	8 (40)	-
ECHOCARDIOGRAPHY			
Aortic valve area, cm^2	2.4±0.6	1.2±0.6	<0.01
Mean pressure gradient, mmHg	4±1	25±16	<0.01
Peak velocity, m/sec	1.4±0.2	3.3±0.9	<0.01
Mean e', cm/s	10.7±4.1	6.5±2.5	<0.01
Median E/e' [IQR]	7.1 [6.0,8.4]	12.3 [8.6,17.0]	0.03
CARDIOVASCULAR MAGNETIC RESONANCE			
Indexed end-diastolic volume (EDVi), mL/m^2	76±14	74±18	0.65
Indexed end-systolic volume, mL/m^2	28±7	23±9	0.07
Stroke volume, mL/m^2	48±8	51±12	0.43
Indexed left ventricular mass (LVMi), g/m^2	67±14	81±18	<0.01
LVMi/EDVi, g/mL	0.88±0.10	1.11±0.20	<0.01

(**Abbreviations**: EDV, end diastolic volume; ESV: end systolic volume; IQR, interquartile range; ACEI angiotensin-converting enzyme inhibitors; ARB angiotensin receptor blockers).

4.4.1 Influence of Heart Rate and Age on T1 Measures

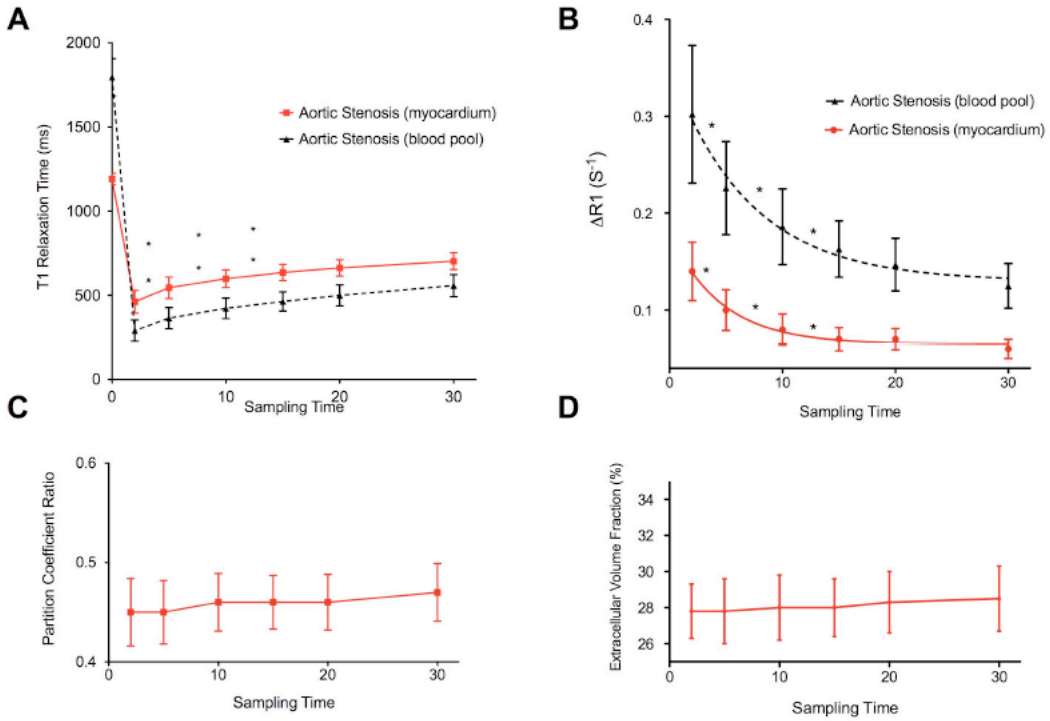
Across the range of heart rates in our study, there was no correlation between heart rate and pre-contrast myocardial T1 (r=-0.23, P=0.16), suggesting that incomplete restoration of magnetization due to fast heart rates and long T1 values was not an important factor. In healthy volunteers (age range 19 to 75) there was no correlation between age and any of the T1 measures investigated (pre-contrast myocardial T1, r=-0.09, P=0.70; post-contrast myocardial T1, r=-0.25, P=0.29; λ, r=0.16, P=0.52; and ECV, r=0.25, P=0.29).

4.4.2 Effects of Time on Post-Contrast T1 Values

Post-contrast T1 values in the blood-pool and myocardium were lower than pre-contrast values, and demonstrated an exponential return to baseline with time (**Figure 4.3** and **Table 4.2**). In particular, the initial 15 min post-contrast was characterized by rapid changes in T1 values but thereafter the relaxation curve appeared to plateau such that subsequent changes were minimal. Indeed, T1 values at 20 min did not differ significantly from those at 15 and 30 min (**Figure 4.3**). On this basis, 20 min was the time-point used for subsequent comparisons.

Interestingly, λ or ECV values were constant at all time-points evaluated, reflecting a constant relationship between the myocardial and blood-pool T1 relaxation times (**Figure 4.3**).

FIGURE 4.3. VARIATION OF DIFFERENT T1 MEASURES WITH TIME



(A) Myocardium and blood pool T1. Post-contrast T1 values are dramatically reduced with the administration of contrast, followed by an exponential increase in values towards baseline. Significant changes in T1 were observed in the first 15 min following contrast administration, while a plateau phase was observed between 15 and 30 min where values remained relatively unchanged. **(B)** Change in T1 relaxation rate (ΔR1) in the myocardium and blood pool. During the first 15 min, significant changes in ΔR1 values were observed in the myocardium and blood pool. This was followed by values that remained relatively stable between 15 and 30 min. **(C and D)** Partition coefficient and extracellular volume fraction. Both measures were constant at all the time points examined, suggesting contrast equilibrium between the blood pool and myocardium occurs as early as 2 min.

* Denotes significant difference in values ($P<0.05$) between two adjacent time points

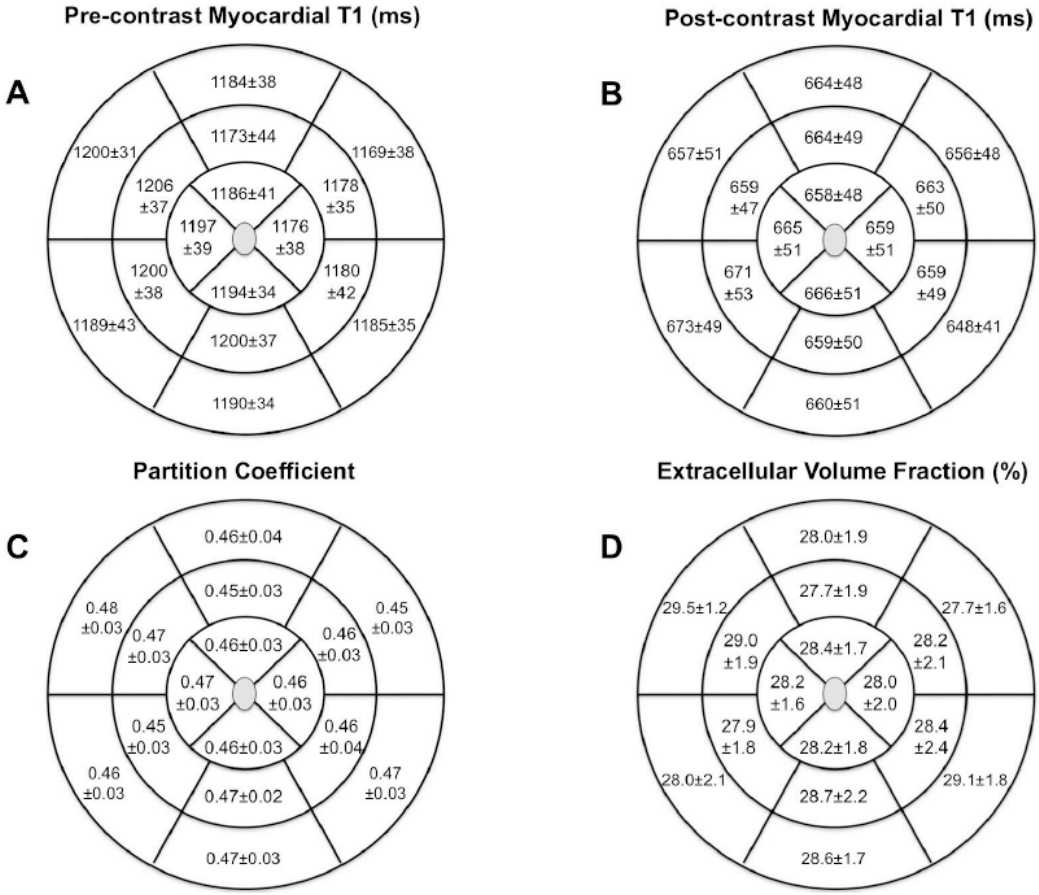
TABLE 4.2. PRE- AND POST-CONTRAST T1, PARTITION COEFFICIENT AND EXTRACELLULAR VOLUME FRACTION VALUES AT ALL TIME POINTS SAMPLED

	Healthy Volunteers	Aortic Stenosis	P value
Pre-contrast			
Myocardial T1, ms	1180±28	1191±34	0.275
Blood-pool T1, ms	1697±89	1796±111	0.003
2 min			
Myocardial T1, ms	451±57	462±68	0.569
Blood-pool T1, ms	271±51	291±63	0.299
Myocardial ΔR1, $ms^{-1}x10^3$	141±30	136±30	0.669
Blood-pool ΔR1, $ms^{-1}x10^3$	323±72	302±71	0.382
λ	0.44±0.04	0.45±0.03	0.207
ECV, %	25.9±2.0	27.8±1.5	0.001
5 min			
Myocardial T1, ms	534±56	545±63	0.556
Blood-pool T1, ms	339±53	365±63	0.173
Myocardial ΔR1, $ms^{-1}x10^3$	105±21	102±21	0.664
Blood-pool ΔR1, $ms^{-1}x10^3$	244±52	226±48	0.261
λ	0.43±0.03	0.45±0.03	0.045
ECV, %	25.5±1.6	27.8±1.8	<0.001
10 min			
Myocardial T1, ms	606±56	599±52	0.671
Blood-pool T1, ms	414±61	423±61	0.661
Myocardial ΔR1, $ms^{-1}x10^3$	82±16	84±16	0.614
Blood-pool ΔR1, $ms^{-1}x10^3$	188±39	186±39	0.865
λ	0.44±0.03	0.46±0.03	0.031
ECV, %	25.8±1.7	28.0±1.6	<0.001
15 min			
Myocardial T1, ms	641±58	636±48	0.765
Blood-pool T1, ms	454±65	464±57	0.621
Myocardial ΔR1, $ms^{-1}x10^3$	73±15	74±12	0.722
Blood-pool ΔR1, $ms^{-1}x10^3$	166±34	163±29	0.764
λ	0.44±0.03	0.46±0.03	0.042
ECV, %	26.0±1.7	28.0±1.6	<0.001
20 min			
Myocardial T1, ms	672±57	663±48	0.585
Blood-pool T1, ms	492±68	500±62	0.703
Myocardial ΔR1, $ms^{-1}x10^3$	65±13	68±11	0.523
Blood-pool ΔR1, $ms^{-1}x10^3$	148±31	147±27	0.909
λ	0.44±0.03	0.46±0.03	0.023
ECV, %	25.9±1.6	28.3±1.7	<0.001
30 min			
Myocardial T1, ms	719±54	703±50	0.361
Blood-pool T1, ms	554±74	558±66	0.859
Myocardial ΔR1, $ms^{-1}x10^3$	55±11	59±10	0.325
Blood-pool ΔR1, $ms^{-1}x10^3$	125±27	125±23	0.933
λ	0.44±0.03	0.47±0.03	0.007
ECV, %	26.2±1.4	28.5±1.8	<0.001

4.4.3 Regional Variation in T1 Measures

In healthy volunteers, there was no variation in any of the T1 measures across the 16 segments of the left ventricle (P>0.1 in all measures; all pairwise comparisons Bonferroni corrected), with similar results demonstrated in patients with aortic stenosis (**Figure 4.4**). Specifically, there were no differences across any of the myocardial slices or between segments within the same slice (P>0.1 for all measures; all pairwise comparisons Bonferroni corrected). Indeed, T1 measures in the mid-cavity were representative of those assessed across the entire left ventricular myocardium (λ 0.46±0.03 *versus* 0.46±0.03, P=1.00; ECV 28.4±1.7 *versus* 28.3±1.9%, P=0.61). Thus, the mid-cavity myocardium was used for subsequent comparisons.

FIGURE 4.4. VARIATION IN THE DIFFERENT T1 MEASURES ACROSS THE LEFT VENTRICULAR MYOCARDIUM IN PATIENTS WITH AORTIC STENOSIS



There were no differences in the pre- (**A**) or post-contrast (**B**) myocardial T1, partition coefficient (**C**), and the extracellular volume fraction (**D**) across the 16 segments. Intra-class coefficients values were >0.90 for each myocardial segment.

Results presented in mean ± standard deviation

4.4.4 Comparison of T1 Measures

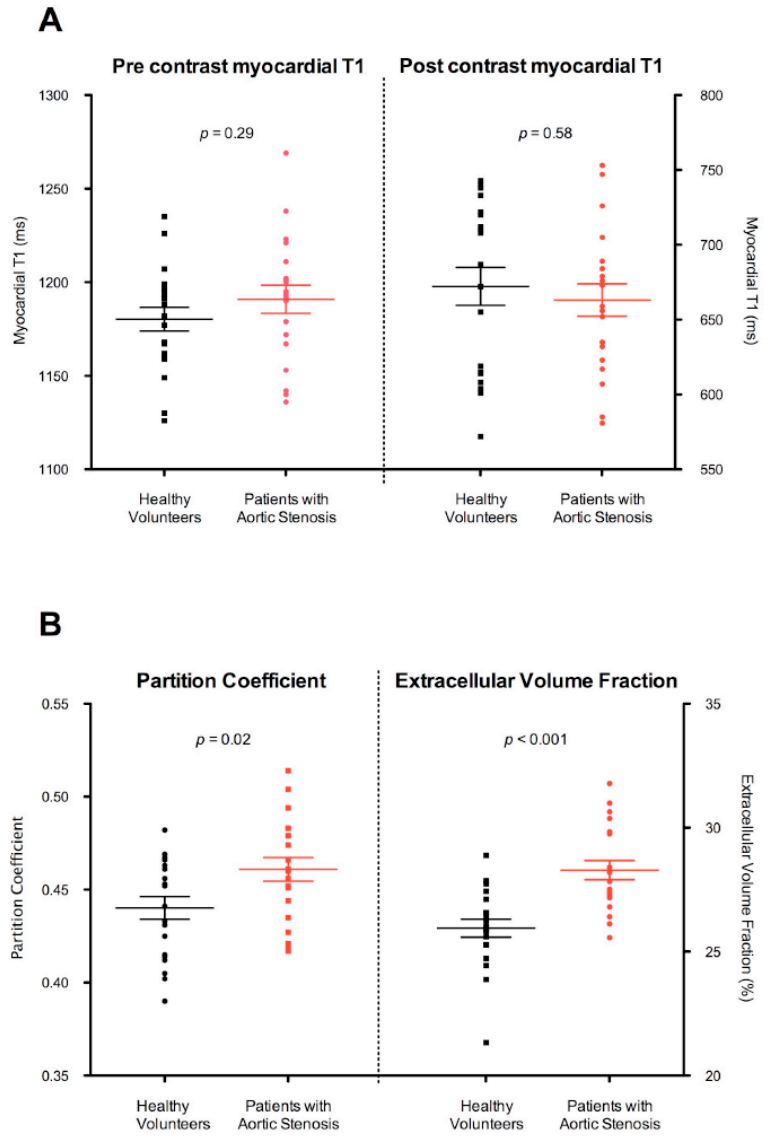
Intra-, and inter-observer reproducibilities were excellent for pre- and post-contrast T1 values, with no fixed or proportional biases and narrow limits of agreement (**Table 4.3**). However, scan-rescan reproducibilities for pre- and post-contrast T1 values were modest (ICC 0.72 and 0.56 respectively). Conversely, all measures of reproducibility were excellent for λ and ECV. ICC values were >0.90 with no fixed biases and narrow confidence limits (**Table 4.3**). The scan-rescan variability was ±3%. Furthermore, the ICC values for both λ and ECV were >0.90 in each of the 16 myocardial segments.

Pre-contrast myocardial T1 values were similar in healthy volunteers and patients with aortic stenosis (1180±28 *versus* 1191±34ms, P=0.29) as were post-contrast T1 values (672±56 *versus* 663±43ms, P=0.59; **Figure 4.5A; Table 4.4**). However, λ and ECV values were higher in patients with aortic stenosis compared to healthy volunteers (λ 0.46±0.03 *versus* 0.44±0.03, P=0.02; ECV 28.3±1.7 *versus* 26.0±1.6%, P<0.001; **Figure 4.5B; Table 4.4**), with the latter appearing to offer better differentiation. The absolute increase in ECV values in patients with aortic stenosis was 2.1% compared to healthy volunteers (95% CI: 0.6 to 3.6%, P=0.009) after adjustment for age and hematocrit levels. Moreover a correlation was observed between ECV and diastolic function (ECV and E/e' r=0.63, P<0.01; ECV and e' r=-0.50, P<0.01), providing indirect support for increased myocardial fibrosis in patients with aortic stenosis. The other T1-related measures did not demonstrate such an association (P>0.05 for all).

TABLE 4.3 REPRODUCIBILITY ANALYSIS

T1 measure	Intra-observer		Inter-observer		Scan-rescan	
	Mean difference (95% CI)	ICC (95% CI)	Mean difference (95% CI)	ICC (95% CI)	Mean difference (95% CI)	ICC (95% CI)
Blood-pool pre-contrast T1, ms	0.6 (-6.0 to 7.2)	1.00 (1.00 to 1.00)	2.6 (-6.1 to 11.3)	1.00 (0.99 to 1.00)	43.6 (-91.7 to 178.9)	0.65 (0.12 to 0.90)
Myocardial pre-contrast T1, ms	-1.8 (-14.7 to 11.1)	0.99 (0.96 to 1.00)	-5.7 (-14.9 to 3.5)	0.99 (0.75 to 1.00)	16.1 (-25.9 to 58.1)	0.72 (0.16 to 0.93)
Blood-pool post-contrast T1, ms	0.4 (-1.7 to 2.5)	1.00 (1.00 to 1.00)	-0.3 (-2.2 to 1.6)	1.00 (1.00 to 1.00)	14.0 (-99.0 to 127.0)	0.58 (-0.02 to 0.88)
Myocardial post-contrast T1, ms	1.4 (-6.7 to 9.5)	1.00 (0.99 to 1.00)	4.5 (-0.7 to 15.5)	1.00 (0.98 to 1.00)	17.8 (-76.9 to 112.5)	0.56 (-0.01 to 0.87)
Partition coefficient	-0.003 (-0.012 to 0.007)	0.99 (0.96 to 1.00)	-0.011 (-0.03 to 0.01)	0.94 (0.31 to 0.99)	-0.003 (-0.02 to 0.02)	0.93 (0.76 to 0.98)
Extracellular volume fraction, %	-0.05 (-0.34 to 0.24)	1.00 (1.00 to1.00)	-0.54 (-1.47 to 0.39)	0.97 (0.60 to 0.99)	0.09 (-1.16 to 1.34)	0.96 (0.85 to 0.99)

FIGURE 4.5. ABILITY OF THE T1 MEASURES TO DIFFERENTIATE PATIENTS WITH AORTIC STENOSIS FROM HEALTHY VOLUNTEERS



(**A**) There was no significant difference in pre- and post-contrast myocardial T1 values between healthy volunteers and patients with aortic stenosis. (**B**) Partition coefficient and extracellular volume fraction values were significantly higher in patients with aortic stenosis compared with healthy volunteers.

Results presented in mean ± standard error of the mean.

TABLE 4.4. MYOCARDIAL T1, PARTITION COEFFICIENT AND EXTRACELLULAR VOLUME FRACTION VALUES OF PATIENTS WITH AORTIC STENOSIS

Patient	Pre-contrast myocardial T1 (ms)	Post-contrast myocardial T1 (ms)	λ	ECV (%)
1	1221	726	0.494	28.2
2	1153	676	0.466	26.1
3	1167	635	0.514	29.8
4	1201	684	0.504	31.8
5	1136	689	0.452	28.4
6	1202	673	0.479	27.8
7	1192	679	0.479	27.2
8	1179	656	0.421	27.4
9	1238	705	0.451	29.8
10	1190	753	0.435	27.3
11	1172	747	0.460	25.6
12	1142	617	0.444	27.2
13	1195	585	0.461	27.5
14	1223	623	0.456	30.6
15	1194	632	0.483	28.2
16	1269	659	0.427	30.4
17	1193	607	0.419	26.4
18	1140	652	0.417	26.8
19	1211	684	0.483	28.2
20	1200	582	0.474	31.0

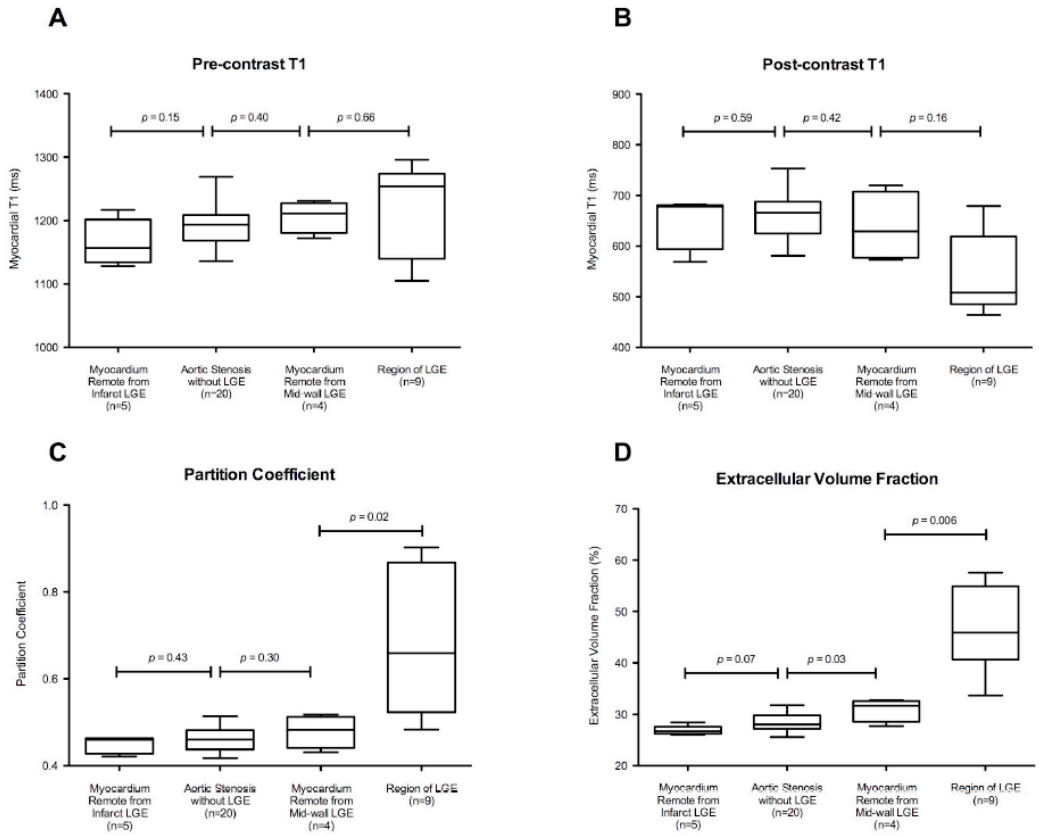
4.4.5 Analysis of T1 Measures in Patients with Focal Late Gadolinium Enhancement

A separate analysis of T1 measures was performed in patients who were excluded because of the presence of focal replacement myocardial fibrosis identified on late gadolinium enhancement (n=9). Regions of interest were placed around areas of late gadolinium enhancement as well as the remote myocardium (defined as an adjacent myocardial segment on the same short axis slice without evidence of late enhancement and then copied onto corresponding pre- and post-contrast T1 maps. T1-related measures were then calculated for both regions.

Among the 9 patients with aortic stenosis and focal late gadolinium enhancement, 5 had an infarct and 4 had a mid-wall pattern of enhancement. The partition coefficient and extracellular volume fraction values were higher in regions of focal enhancement compared to the myocardium remote to the mid-wall enhancement (λ 0.69±0.16 *versus* 0.48±0.04, P=0.02; ECV 46.6±8.5% *versus* 30.9±2.3%, P=0.006) but surprisingly, there was no difference in pre- and post-contrast T1 values (**Figure 4.6**). Furthermore, extracellular volume fraction values were also increased in the remote myocardium of patients with mid-wall late gadolinium enhancement compared to those without late enhancement (30.9±2.7 *versus* 28.3±1.7%, P<0.01). This was not observed with respect to pre- and post-contrast T1 nor partition coefficient values.

The study was not powered to investigate T1-related measures in the remote myocardium in patients with mid-wall late enhancement. However, the increased extracellular volume fraction values in these regions support the concept that mid-wall fibrosis represents the end-stage of the myocardial fibrotic response.

FIGURE 4.6. ANALYSIS OF T1 MEASURES IN PATIENTS WITH LATE GADOLINIUM ENHANCEMENT



(**A**) and (**B**) Pre- and post-contrast T1 values were similar in all myocardial regions examined. (**C**) Partition coefficient values were highest in regions of focal late gadolinium enhancement (LGE). The values were similar in other regions. (**D**) Extracellular volume fraction was highest in regions of focal LGE. Compared to patients with aortic stenosis without mid-wall LGE, extracellular volume fraction was higher in the remote myocardium of those patients with mid-wall LGE.

Results were presented in box-and-whiskers plots (Tukey): the central box represents the interquartile range, with the median indicated by the line within the box. The whiskers extend to the most extreme values within the 1.5 interquartile ranges.

4.5 DISCUSSION

Using a standardized methodology and patient cohort, we have systematically compared commonly used T1 measures at multiple time-points and in multiple regions across the left ventricle. We have shown that pre-contrast T1 was limited by an inability to differentiate patients with aortic stenosis from healthy volunteers, while post-contrast myocardial T1 lacked sufficient scan-rescan reproducibility. By comparison, partition coefficient and in particular extracellular volume fraction demonstrated excellent reproducibility and were increased in patients with aortic stenosis compared to healthy volunteers.

Over recent years, multiple T1-derived parameters have been derived to assess diffuse myocardial fibrosis. We have attempted to optimize and compare these different techniques at 3T using a standardized technique with meticulous attention paid to avoid blood-pool and artifact. This approach demonstrated excellent reproducibility, with respect to the entire ventricle and within individual myocardial segments, indicating that both global and regional T1 can be measured. Furthermore, pre- and post-contrast T1, partition coefficient and extracellular volume fraction values did not vary across segments in the left ventricle. This is as anticipated given the diffuse distribution of interstitial fibrosis.

We have also characterized the temporal variation of T1 measures. Post-contrast T1 demonstrated the characteristic exponential increase back to baseline following gadolinium administration. The rate of this recovery appears to be determined by the blood concentration of gadolinium (equilibrium between blood-pool and myocardial T1 occurred as early as 2 min), which depends on its volume of distribution and renal clearance. Whilst the first 15 min following injection were characterized by large variations in myocardial and blood-pool T1 values, a plateau phase ensued between 15 and 30 min during which T1 values were relatively constant. We therefore investigated whether meaningful comparisons could be made between serial scans using post-contrast T1 values at 20 min. Unfortunately

the scan-rescan reproducibility of post-contrast T1 was modest. This is likely the consequence of inter-day variation in gadolinium pharmacokinetics relating to glomerular filtration rate, the patient's volume status and diet. Such variation will have had a major impact on our post-contrast T1 values potentially obscuring any differences attributable to diffuse fibrosis. Indeed this is the likely explanation for the lack of difference in values between patients with aortic stenosis and healthy volunteers. Whilst complex kinetic models have been developed in an attempt to correct for some of these factors these are based upon multiple assumptions and data acquired at 1.5 not 3T (165).

An alternate T1 technique is therefore necessary to overcome variations in gadolinium kinetics. One approach is pre-contrast myocardial T1. In this study, pre-contrast myocardial T1 demonstrated improved scan-rescan reproducibility; but, like post-contrast T1, was unable to differentiate patients with aortic stenosis from healthy volunteers. This probably reflects the reduced sensitivity of pre-contrast techniques that rely on the inherent T1 properties of healthy myocardium and fibrosis. By comparison a recent study demonstrated that pre-contrast myocardial T1 values (using the shortened modified MOLLI sequence) were higher in patients with severe aortic stenosis compared to healthy volunteers (147). Most likely this reflects the more advanced disease state in their study population. Indeed there was no increase in values amongst their patients with moderate stenosis, who had a similar degree of hypertrophy to our cohort (81±18 *versus* 82±17 g/m^2).

A second approach is to correct post-contrast myocardial T1 values for variation in the pharmacokinetics of gadolinium. Partition coefficient appears to be an effective method by using a ratio of myocardial and blood-pool T1 values. Indeed, partition coefficient demonstrated excellent reproducibility, indicating that inter-day variation in gadolinium kinetics can be accounted for by this approach. Furthermore, partition coefficient values are increased in patients with aortic stenosis compared to healthy volunteers. This probably reflects the improved sensitivity of contrast-enhanced

techniques, based on the accumulation of gadolinium in regions of fibrosis and the resultant shortening of T1 (44).

Extracellular volume fraction translates partition coefficient into a percentage of the myocardium affected by diffuse fibrosis and is in many ways easier to conceptualize. Furthermore, it corrects for the effects of plasma volume, which can vary considerably from day to day (perhaps accounting for some of the scan-rescan variation in pre- and post-contrast T1 values). Extracellular volume fraction demonstrated excellent reproducibility in this study and appears to further improve the differentiation between patients with aortic stenosis and healthy volunteers.

Both partition coefficient and extracellular volume fraction appear to possess all the necessary attributes for the measurement of diffuse fibrosis at 3T. Interest surrounds such techniques in the assessment of novel anti-fibrotic agents in aortic stenosis (3) and our scan-rescan reproducibility will be of use when estimating the required sample sizes for such studies. These will require the excellent reproducibility provided by partition coefficient and extracellular volume fraction to ensure that any difference detected between scans is attributed to the intervention. Moreover, the slowly progressive nature of fibrosis means that any treatment differences are likely to be small, so that the improved sensitivity of partition coefficient and extracellular volume fraction in particular over pre-contrast T1 will also be important.

4.5.1 Study Limitations

By design we did not recruit patients with end-stage aortic stenosis, as we believe that T1 mapping will be more relevant to those with less severe disease in whom myocardial fibrosis is more likely to be reversible with novel anti-fibrotic therapies. Unfortunately, this has limited our ability to validate the various T1 measures against histology. However, good correlations for each technique have previously been established with histology, and supported in this study by increased extracellular volume fraction and partition coefficient in regions of late gadolinium enhancement. Furthermore, we have demonstrated a close association between extracellular volume fraction and markers of diastolic dysfunction. It is therefore reasonable to assume that our T1-derived measures are markers of diffuse myocardial fibrosis particularly as we have carefully excluded individuals with pathologies that might have confounded our results.

The patients with aortic stenosis were significantly older than healthy volunteers in our study, but the differences in extracellular volume fraction and partition coefficient values between patients with aortic stenosis and healthy volunteers were independent of age. This is also consistent with previous studies (152,166).

Scan-rescan reproducibility was only assessed in healthy volunteers, but not patients with aortic stenosis. However, both patients with aortic stenosis and healthy volunteers demonstrated similar T1 relaxation profiles in the myocardium and blood-pool. Furthermore, we did not observe any proportional bias in healthy volunteers. Therefore, we are confident the scan-rescan reproducibility in patients with aortic stenosis should be similar to that in healthy volunteers.

4.6 CONCLUSIONS

In the cardiovascular magnetic resonance assessment of diffuse myocardial fibrosis, pre- and post-contrast myocardial T1 have limitations. By contrast, partition coefficient and in particular extracellular volume fraction demonstrate excellent reproducibility and an ability to differentiate patients with aortic stenosis from healthy volunteers. Among all the T1-derived measures evaluated, extracellular volume fraction appears to have the most potential in assessing diffuse myocardial fibrosis at 3T.

CHAPTER 5

HIGH-SENSITIVITY TROPONIN I CONCENTRATIONS ARE A MARKER OF AN ADVANCED HYPERTROPHIC RESPONSE AND ADVERSE OUTCOMES IN PATIENTS WITH AORTIC STENOSIS

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* Equal contributions as first authors

5.1 SUMMARY

AIMS: High-sensitivity cardiac troponin I (cTnI) assays hold promise in detecting the transition from hypertrophy to heart failure in aortic stenosis. We sought to investigate the mechanism for troponin release in patients with aortic stenosis and whether plasma cTnI concentrations are associated with long-term outcome.

METHODS: Plasma cTnI concentrations were measured in two patient cohorts using a high-sensitivity assay. First, in the Mechanism Cohort, 122 patients with aortic stenosis (median age 71, 67% male, aortic valve area 1.0±0.4cm^2) underwent cardiovascular magnetic resonance and echocardiography to assess left ventricular (LV) myocardial mass, function and fibrosis. In the separate Outcome Cohort, 131 patients originally recruited into the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact of REgression (SALTIRE) study, had long-term follow up for the occurrence of aortic valve replacement and cardiovascular deaths.

RESULTS: The indexed LV mass and measures of replacement fibrosis (late gadolinium enhancement) were associated with cTnI concentrations independent of age, sex, coronary artery disease, aortic stenosis severity and diastolic function. Over a median follow-up of 10.6 years (1,178 patients-years), 24 patients died from a cardiovascular cause and 60 patients had an aortic valve replacement. Plasma cTnI concentrations were associated with aortic valve replacement or cardiovascular death (HR 1.77; 95% CI 1.22-2.55) independent of age, sex, systolic ejection fraction, and aortic stenosis severity.

CONCLUSIONS: In patients with aortic stenosis, plasma cTnI concentration is associated with advanced hypertrophy and replacement myocardial fibrosis as well as AVR or cardiovascular death.

5.2 INTRODUCTION

Aortic stenosis is the commonest form of valvular heart disease in the western world and its prevalence is expected to double in the next two decades (1). Current guidelines advocate aortic valve replacement in patients with symptoms and severe valve narrowing (5,141). However, there is a poor correlation between the severity of stenosis and symptom onset making the management of asymptomatic patients controversial (5). This apparent discrepancy might in part be explained by heterogeneity in the hypertrophic response to aortic stenosis, which itself is an independent marker of an adverse prognosis (3,53,58).

Hypertrophy occurs in response to the increased afterload imposed by aortic valve narrowing on the left ventricle. Initially, this restores wall stress and maintains cardiac performance but decompensation ultimately ensues and patients develop symptoms, adverse events and the need for surgery. The transition from hypertrophy to heart failure is characterized by progressive cardiomyocyte death and replacement fibrosis (36). Myocardial fibrosis can be detected using two cardiovascular magnetic resonance techniques: late gadolinium enhancement (replacement fibrosis) and T1 mapping (diffuse interstitial fibrosis) with data indicating that the former provides useful prognostic information (51,58). However to date, a marker of myocyte cell death has been lacking.

Cardiac troponin is a structural protein present in cardiac muscle, with plasma troponin concentrations considered a highly specific marker for myocardial injury (115). Recent advances in assay technology have greatly improved sensitivity, now allowing quantification of troponin with a high degree of precision at extremely low plasma concentrations (116).

In this study, we hypothesized that detection of myocardial injury by high-sensitivity troponin assays may provide an early indicator of left ventricular decompensation and be associated with future adverse events in patients with aortic stenosis.

5.3 METHODS

Two cohorts of stable patients with aortic stenosis were recruited from cardiology outpatient clinics across 3 centers in Southeast Scotland. First we determined the association between plasma cardiac troponin I (cTnI) concentrations and left ventricular functional and structural abnormalities on cardiovascular magnetic resonance (Mechanism Cohort), and second we examined the prognostic role of plasma cTnI concentrations in patients with aortic stenosis (Outcome Cohort). The study was conducted in accordance with the Declaration of Helsinki, and approved by the local research ethics committee. Written informed consent was obtained from all participants.

5.3.1 Patient Populations

Mechanism Cohort: Patients in the Mechanism Cohort were recruited prospectively as stated in Chapter 2. In addition, thirteen age- and sex-matched healthy volunteers without clinically significant heart disease were recruited from the local community.

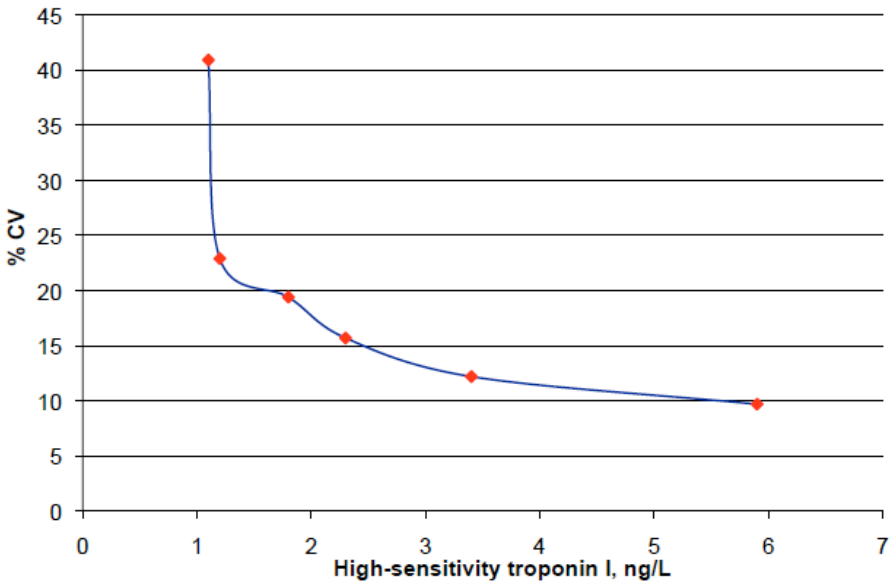
Outcome Cohort: This cohort comprised patients recruited into the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact of REgression (SALTIRE) study. The study design, inclusion and exclusion criteria have been described previously (127). In brief, from March 2001 to April 2002, 155 patients with asymptomatic aortic stenosis were randomly assigned to receive either atorvastatin 80 mg or placebo once daily. Patients were excluded if they were already on a statin, if aortic valve replacement was planned or if they had moderate or severe left ventricular systolic impairment. Only patients with plasma samples available for cTnI analysis were included in the present analysis.

5.3.2 Blood Sampling and Analysis

In the Mechanism Cohort, brain natriuretic peptide (BNP) concentration was analyzed with the Triage BNP assay (Biosite, Inc., San Diego, California). The inter-assay coefficient of variation was 10% at 28.8 pg/ml, with a detection range of 5 to 1300 pg/ml (167). In the Outcome Cohort, N-terminal proBNP (NT-proBNP) concentration was measured using the Elecysys 2010 analyzer (Roche Diagnostics Ltd, Lewes, UK). This assay has <0.001% cross-reactivity with bioactive BNP, and the inter-assay coefficients of variation range from 0.9 to 5.5% (168).

Plasma cTnI concentrations were determined by the ARCHITECT *STAT* high-sensitivity cTnI assay (Abbott Laboratories, Abbott Park, Illinois) in both cohorts. The method of detection and sensitivity of the assay have been provided in greater detail in Chapter 2. Precision profiling of the assay was performed in 248 samples across 18 healthy controls (**Figure 5.1**). Concentrations lower than the detection limit were assigned a value of 1.2 ng/L.

FIGURE 5.1. ANALYTICAL VARIABILITY OF HIGH-SENSITIVITY CARDIAC TROPONIN I ASSAY



Precision profiling of the ARCHITECT$_{STAT}$ high-sensitivity troponin I assay was performed in 248 samples across 18 healthy controls. The inter-assay coefficient of variation (CV) for duplicate samples is 10% at 6 ng/L and 20% at 1.5 ng/L.

5.3.3 Imaging Protocol in the Mechanism and Outcome Cohorts

All participants in both cohorts underwent echocardiography to assess aortic stenosis severity and diastolic function according to the protocol described in Chapter 2. Patients in the Mechanism Cohort also underwent cardiovascular magnetic resonance at 3T to assess left ventricular volumes, mass and function. Furthermore, assessment of myocardial fibrosis was performed using both conventional late gadolinium enhancement and novel myocardial T1 mapping techniques as detailed in Chapter 2.

In addition to echocardiography, patients in the Outcome Cohort also underwent computed tomography calcium scoring of the coronary arteries and aortic valve was performed on ECG-gated non-contrast scans using a double helix scanner (Twin II Flash, Philips Medical Systems). All images were analyzed by a single operator using the Picker Cardiac Scoring software as previously described (127).

5.3.4 Follow-up in the Outcome Cohort

Clinical outcomes were obtained and adjudicated by two independent investigators blinded to plasma cTnI and BNP concentrations. All in-hospital and community deaths were captured in a comprehensive national database: the General Register of Scotland. Cardiovascular death was based on the cause of death stated on the death certificate. We defined cardiovascular death as death due to myocardial infarction, sudden cardiac death, heart failure, stroke, death due to cardiovascular procedures and death due to other cardiovascular causes. Each death was classified as cardiac or non-cardiac by two independent investigators and any discrepancy resolved by consensus. All events were confirmed by independent review of each patient's electronic healthcare record where available. Surgical aortic valve replacement (no patients underwent transcatheter aortic valve implantation in the follow-up period) was determined from individual patient medical records. All patients in the Outcome Cohort were managed in the tertiary cardiac center, where patients are reviewed at a multi-disciplinary meeting prior to undergoing cardiac surgery. Only patients with established indications were referred for aortic valve replacement according to the European Society of Cardiology recommendations (5,169).

5.3.5 Statistical Analysis

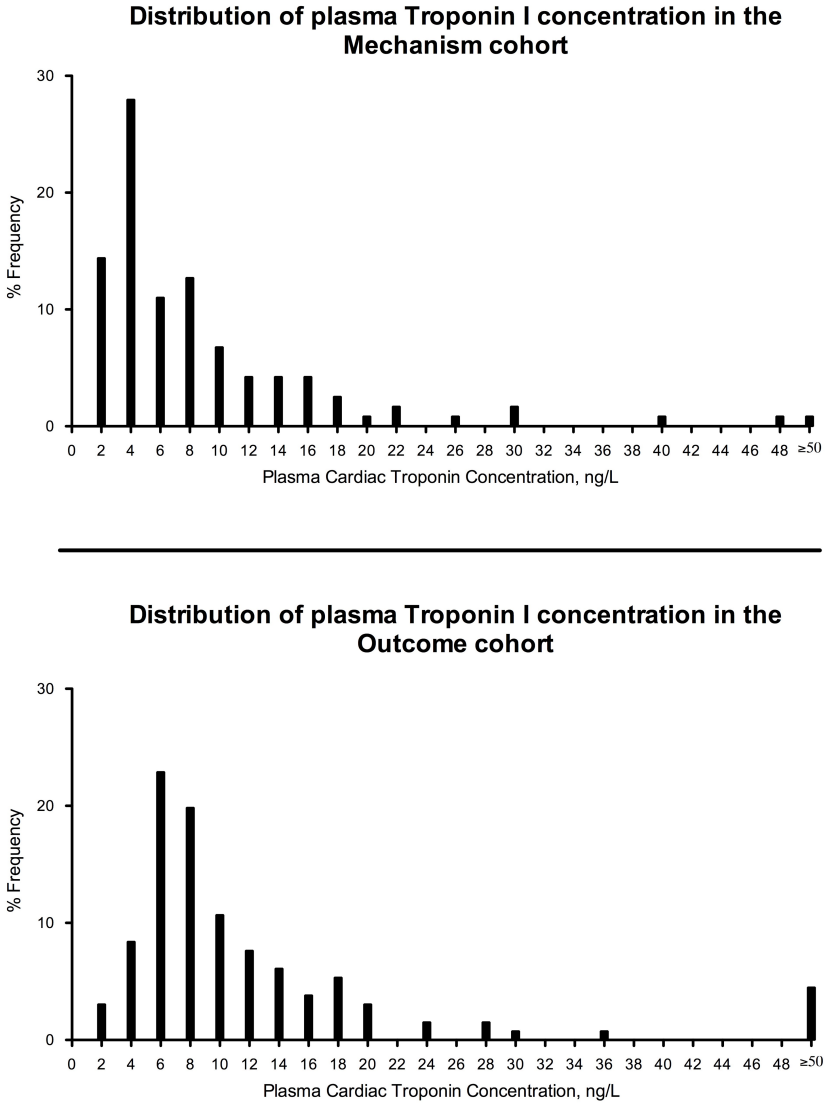
We assessed the association of plasma cTnI concentrations with measures of aortic stenosis and ventricular remodeling using univariate and multivariable linear regression models. Plasma cTnI concentrations were log-transformed as this variable was highly skewed. Kaplan-Meier analysis was performed across tertiles of cTnI concentrations. To accommodate competing risks, the association between time to aortic valve replacement or cardiovascular deaths and plasma cTnI concentrations (log-transformed [base 2]) was modeled as a composite endpoint in Cox proportional hazard models. Furthermore, we examined whether relative change in cTnI concentrations at 1 year (cTnI at 1 year – baseline cTnI, both log transformed) was associated with increased odds of an event at 3-year, and 5-year follow-up independent of baseline cTnI concentrations. Survival analyses and all other analyses were performed using R version 2.15.2 (Vienna, Austria) and SPSS Version 19 (SPSS, Inc., Chicago, IL, USA), respectively.

5.4 RESULTS

We recruited 122 patients into the Mechanism Cohort (71 [65-77] years, 67% males, aortic valve area 1.0±0.4 cm^2) and analyzed 131 patients in the Outcome Cohort (69 [62-75] years, 70% males, aortic valve area 1.1±0.4 cm^2) (**Tables 5.1 and 5.2**). Thirteen healthy volunteers were recruited, who were well matched in terms of age (65 [57-75] years) and sex (62% male) compared to the other groups and did not have any history of diabetes mellitus, hypertension or coronary artery disease.

Plasma cTnI concentrations above the lower limit of detection of 1.2 ng/L were present in 98% of our patients with aortic stenosis and increased in both cohorts compared to the healthy volunteers (Mechanism Cohort 6.6 [3.8-12.0] ng/L; Outcome Cohort 7.6 [5.7-13.2] ng/L; healthy volunteers 3.2 [1.3-11.0] ng/L). There were 10 patients (8.1%) in the Mechanism Cohort and 10 patients (7.6%) in the Outcome Cohort with plasma cTnI concentrations above 26 ng/L (the 99^{th} centile derived from the healthy reference population). There was no difference in renal function across tertiles of cTnI in patients with aortic stenosis (**Figure 5.2**).

FIGURE 5.2. DISTRIBUTION OF HIGH-SENSITIVITY PLASMA CARDIAC TROPONIN I CONCENTRATIONS IN THE MECHANISM AND OUTCOME COHORTS OF PATIENTS WITH AORTIC STENOSIS



Plasma cardiac troponin I concentrations were above the limit of detection in 98% of our patients with aortic stenosis in both cohorts, and exceeded the recommended diagnostic threshold for myocardial infarction (> 26ng/L) in 8%. The distribution of plasma troponin I concentrations were similar in the two cohorts of patients.

TABLE 5.1. BASELINE CHARACTERISTICS OF PATIENTS WITH AORTIC STENOSIS IN THE MECHANISM COHORT

	Healthy Volunteers (n=13)	Mechanism Cohort (n=122)	P value
CLINICAL CHARACTERISTICS			
Age, years	65 [57,75]	71 [65,77]	0.13
Male sex, n (%)	8 (62)	82 (67)	0.76
Diabetes mellitus, n (%)	0	14 (11)	-
Hypertension, n (%)	0	78 (63)	-
Coronary artery disease, n (%)	0	41 (33)	-
SBP, mmHg	148±12	149±20	0.35
NYHA class, n (%)			
I	13 (100)	63 (52)	-
II	0	35 (28)	
III	0	24 (20)	
Creatinine, μmol/l	69±8	78±17	0.06
Cardiac troponin I concentration, ng/L	3.2 [1.3,11.0]	6.6 [3.8,12.0]	0.03
Brain natriuretic peptide, pg/ml	10.3 [5.6,18.1]	26.4 [10.6,53.9]	0.009
ECHOCARDIOGAPHY			
Peak aortic jet velocity, m/s	1.4±0.2	3.7±0.9	<0.001
Mean pressure gradient, mmHg	4±1	32±18	<0.001
Aortic valve area, cm^2	2.4±0.7	1.0±0.4	<0.001
Valvulo-arterial impedance, mmHg/ml•m^2	4.5±1.1	4.5±1.2	0.96
Mean e', cm/s	8.1±2.7	6.2±1.9	0.001
Mean E/e'	7.9±2.2	14.8±8.1	0.003
CARDIOVASCULAR MAGNETIC RESONANCE			
Indexed end-diastolic volume (EDVi), ml/m^2	73±13	72±14	0.71
Indexed end-systolic volume, ml/m^2	27±7	24±9	0.28
Indexed stroke volume, ml/m^2	46±7	48±9	0.68
Ejection fraction, %	64±3	67±7	0.12
Indexed left ventricular mass (LVMi), g/m^2	70±14	89±22	0.004
LVMi/EDVi, g/ml	0.96±0.13	1.26±0.28	<0.001
Extracellular volume fraction, %	25.9±1.6	27.7±2.5	0.01

TABLE 5.2. CHARACTERISTICS OF PATIENTS IN THE OUTCOME COHORT

	All patients (n=131)	Tertile 1 ≤6.3 ng/L (n=42)	Tertile 2 6.4–10.6 ng/L (n=45)	Tertile 3 ≥10.7 ng/L (n=44)	P value
CLINICAL CHARACTERISTICS					
Age, years	67±10	64±12	69±10	70±9	0.03
Male sex, n (%)	91 (70)	24 (57)	32 (71)	35 (79)	0.08
Diabetes Mellitus, n (%)	4 (3)	1 (2)	1 (2)	2 (5)	-
Hypertension, n (%)	66 (50)	18 (43)	22 (49)	26 (59)	0.31
Coronary artery disease, n (%)	22 (16)	6 (14)	7 (16)	9 (21)	0.72
Systolic blood pressure, mmHg	145±20	139±17	148±21	146±19	0.07
NYHA class, n (%)					
I	117 (89)	38 (90)	41 (91)	38 (86)	0.53
II	14 (11)	4 (10)	4 (9)	6 (14)	
Creatinine, μmol/l	91±21	86±17	92±20	95±25	0.12
NT-pro-BNP, pg/ml	198.0 [113.5,530.5]	129.5 [76.3,228.0]	180.0 [89.0,416.0]	507.0 [181.5,1103.0]	0.008
ECHOCARDIOGRAPHY					
Peak aortic jet velocity, m/s	3.4±0.7	3.4±0.6	3.4±0.6	3.5±0.7	0.45
Mean pressure gradient, mmHg	26±11	25±10	25±10	28±13	0.35
Aortic valve area (AVA), cm^2	1.1±0.4	1.0±0.4	1.1±0.4	1.0±0.4	0.72
Indexed AVA, cm^2/m^2	0.5±0.2	0.5±0.2	0.6±0.2	0.5±0.2	0.66
Left ventricular mass (LVM), g	357±107	327±111	350±102	393±100	0.02
Indexed LVM, g/m^2	180±50	165±54	172±49	196±49	0.06
Fractional shortening, %	40±8	42±9	42±8	37±6	0.004
Ejection fraction (EF), %	70±10	72±11	72±9	66±8	0.007
Left ventricular hypertrophy, n (%)	109 (95)	34 (81)	36 (80)	39 (89)	0.49
Impaired EF <50%, n (%)	4 (3)	1 (2)	2 (4)	2 (5)	0.84
COMPUTED TOMOGRAPHY					
Coronary calcium score, log AU	1.6±1.3	1.5±1.3	1.5±1.3	1.8±1.1	0.53
Aortic valve calcium score, log AU	3.6±0.6	3.6±0.5	3.6±0.5	3.7±0.8	0.61

5.4.1 Mechanism for Increased Cardiac Troponin I Concentrations

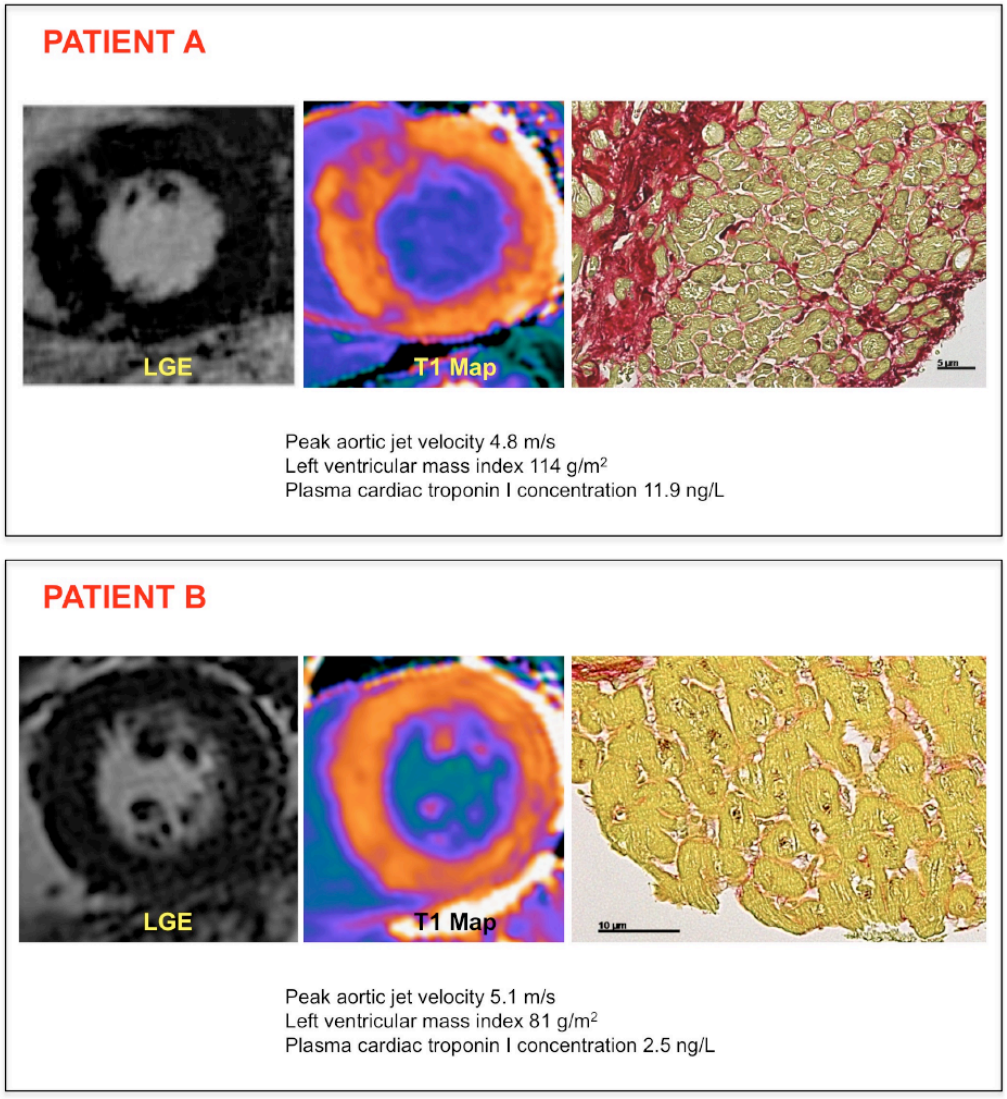
In the Mechanism Cohort, patients with aortic stenosis had an increased left ventricular mass index compared to healthy controls, although there was no difference in left ventricular volumes or ejection fraction (**Table 5.1**). Furthermore, these patients had higher extracellular volume fraction values (27.7±2.5 versus 25.9±1.6%, P=0.01), and 35 patients (28%) had a mid-wall pattern of late gadolinium enhancement: an observation not seen among the healthy volunteers (**Figure 5.3**).

Plasma cTnI concentrations correlated with left ventricular mass index, independent of coronary artery disease status (r=0.50, P<0.001; **Figure 5.4**). A weaker correlation was also observed between plasma cTnI concentrations and peak aortic jet velocity (r=0.32, P<0.001). Furthermore, patients with aortic stenosis and mid-wall late gadolinium enhancement had a two-fold increase in plasma cTnI concentrations compared to those without (9.5 [5.7, 20.3] ng/L *versus* 4.3 [3.3, 7.9] ng/L, P=0.02; **Figure 5.5**).

With univariate analysis, age, mean pressure gradient, mean e', the left ventricular mass index, and measures of both diffuse and replacement fibrosis were all associated with plasma cTnI concentrations (**Table 5.3**; all P<0.05). However, only age, left ventricular mass index and extent of late gadolinium enhancement (%) were independently associated with plasma cTnI concentrations (**Model 1; Table 5.3**).

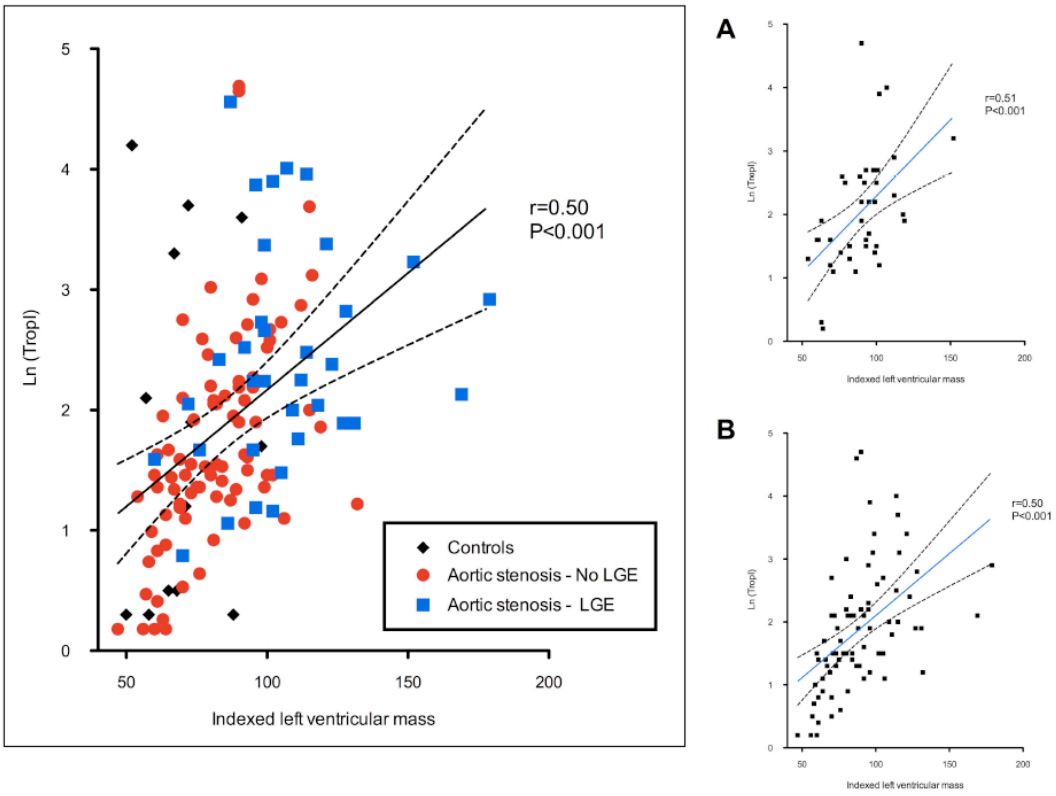
Interestingly, there was no difference in plasma cTnI concentrations between patients with and without coronary artery disease (6.9 [4.0, 13.5] ng/L *versus* 6.2 [3.5, 10.0] ng/L, P=0.28). This was supported by data from the Outcome Cohort where no correlation was observed between the coronary calcium scores and plasma cTnI concentrations (r=-0.03, P=0.71).

FIGURE 5.3. COMPARISON OF TWO PATIENTS WITH SEVERE AORTIC STENOSIS



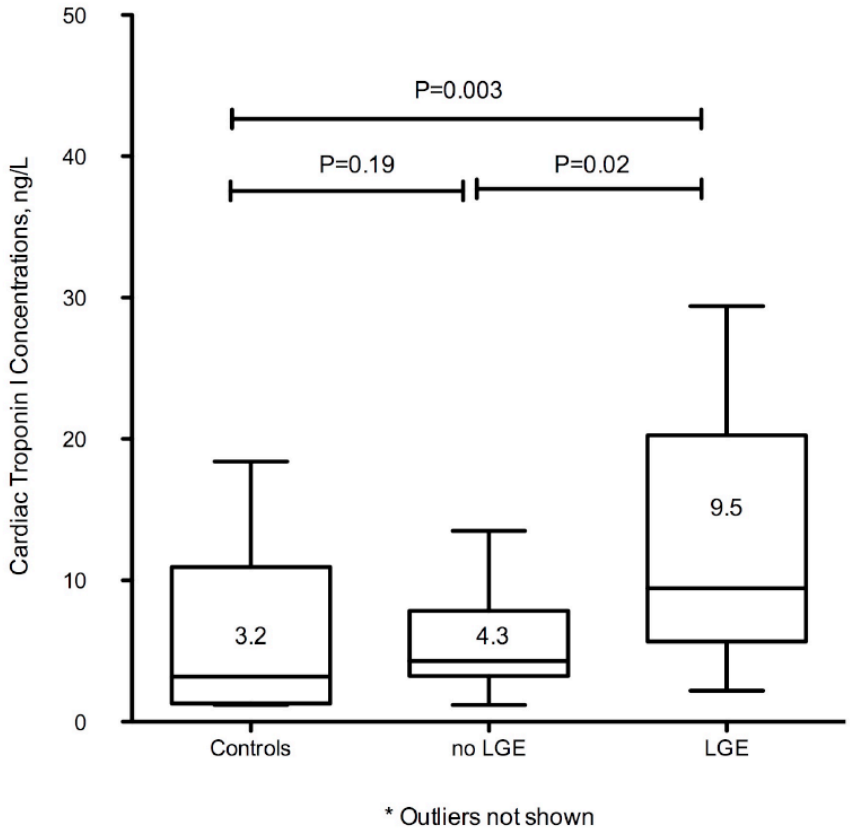
Both had similar severity of aortic valve narrowing (peak aortic jet velocity in **Patient A** was 4.8 m/s and **Patient B** was 5.1 m/s) and neither had significant coronary artery disease. However, high-sensitivity troponin I concentration was more than four-fold higher in **Patient A** (11.9 ng/L) compared to **Patient B** (2.5 ng/L), consistent with more advanced hypertrophy (left ventricular mass index in **Patient A** was 114 g/m^2 and **Patient B** was 81 g/m^2). Furthermore, **Patient A** had evidence of focal mid-wall fibrosis on late gadolinium enhancement (LGE) and myocardial T1 mapping, as well as, more extensive collagen deposition on histology (picrosirius red stains).

FIGURE 5.4. CORRELATION BETWEEN INDEXED LEFT VENTRICULAR MASS AND PLASMA CARDIAC TROPONIN I CONCENTRATIONS



Similar correlations was seen in patients with (**A**) and without (**B**) coronary artery disease (troponin I concentrations were log-transformed).

FIGURE 5.5. CARDIAC TROPONIN I CONCENTRATIONS IN PATIENTS WITH AND WITHOUT MID-WALL FIBROSIS



Patients with aortic stenosis and mid-wall late gadolinium enhancement (LGE) had a two-fold increase in cardiac troponin I concentrations compared with those without LGE and age- and sex-matched healthy patients.

Results were presented in box-and-whiskers plots (Tukey): the central box represents the interquartile range, with the median indicated by the line within the box. The whiskers extend to the most extreme values within the 1.5 interquartile ranges.

TABLE 5.3. UNIVARIATE AND MULTIVARIABLE LINEAR REGRESSION ANALYSIS TO EXAMINE ASSOCIATION OF VARIABLES WITH PLASMA CARDIAC TROPONIN I CONCENTRATIONS

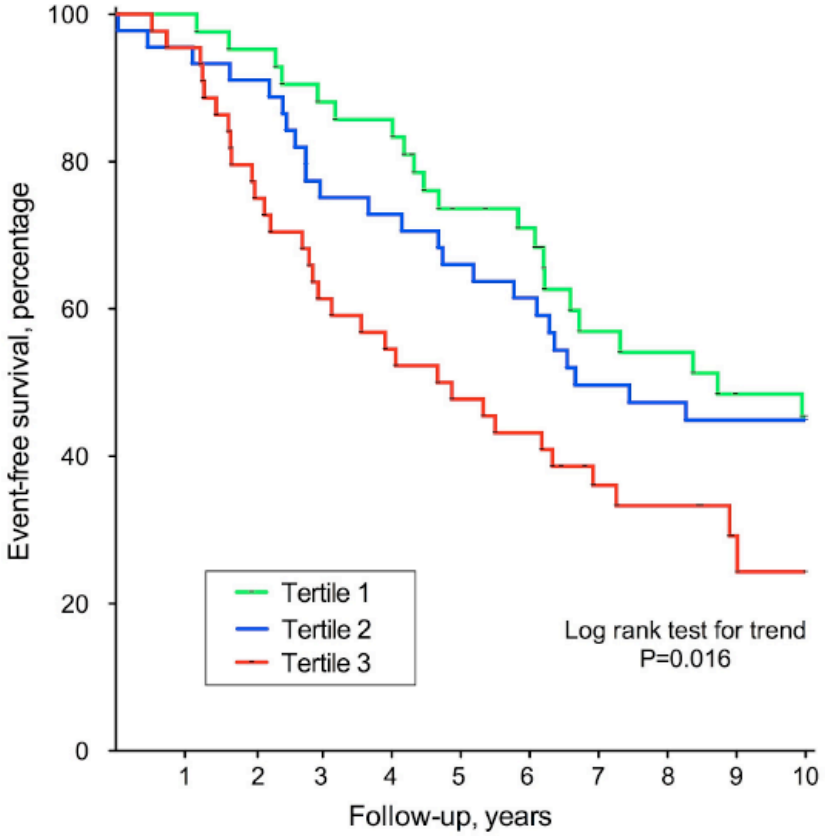
Variables	Univariate		Multivariable – Model 1 (Included %LGE)		Multivariable – Model 2 (Included ECV)	
	Relative change in troponin I concentration (95% CI)	P value	Relative change in troponin I concentration (95% CI)	P value	Relative change in troponin I concentration (95% CI)	P value
Age, per 10 years	1.32 (1.07 to 1.44)	0.004	1.49 (1.14 to 1.80)	0.002	1.36 (1.09 to 1.72)	0.006
Male sex	1.31 (0.90 to 1.92)	0.16	0.79 (0.44 to 1.42)	0.44	0.80 (0.46 to 1.39)	0.42
Diabetes mellitus	0.92 (0.52 to 1.61)	0.76				
Hypertension	1.21 (0.83 to 1.74)	0.33				
Coronary artery disease	1.20 (0.82 to 1.75)	0.35	1.01 (0.58 to 1.73)	0.96	1.17 (0.72 to 1.91)	0.53
Mean pressure gradient, per 10 mmHg	1.17 (1.02 to 1.35)	0.02	0.92 (0.79 to 1.06)	0.27	0.93 (0.73 to 1.06)	0.28
Mean e', cm/s	0.86 (0.78 to 0.95)	0.002	1.08 (0.93 to 1.27)	0.30	1.02 (0.88 to 1.19)	0.78
Indexed left ventricular mass, per 10 g/m^2	1.23 (1.15 to 1.32)	<0.001	1.34 (1.15 to 1.55)	<0.001	1.41 (1.23 to 1.62)	<0.001
Extent of late gadolinium enhancement (%LGE), %	1.13 (1.08 to 1.17)	<0.001	1.11 (1.03 to 1.19)	0.006		
Extracellular volume fraction (ECV), %	1.15 (1.07 to 1.23)	<0.001			1.11 (1.00 to 1.21)	0.05

5.4.2 Prognostic Value of Cardiac Troponin I Concentrations

Patients in the Outcome Cohort were stratified by tertiles of plasma cTnI concentration (**Table 5.2**). In comparison to the lowest tertile, patients in the highest tertile were older (70±9 *versus* 64±12 years, P=0.03) and had an increased ventricular mass (393±100 *versus* 327±111g, P=0.02). However, there were no differences in co-morbidity, severity of aortic stenosis or coronary calcium scores across the tertiles (P>0.1 for all; **Table 5.2**).

Over a median of 10.6 years follow-up (1,178 patient-years), 60 patients had an aortic valve replacement, 24 died from a cardiovascular cause and 47 died from non-cardiovascular causes. Ten-year event-free survival rate for aortic valve replacement or cardiovascular deaths differed across the tertiles of cTnI concentrations (log rank test for trend, P=0.016, **Figure 5.6**). Plasma cTnI concentration was associated with an increased risk of aortic valve replacement or cardiovascular deaths in unadjusted analysis (HR 1.65 per two-fold increment in cTnI concentration; 95%CI 1.15 to 2.38, P=0.007) with minimal attenuation in the effect estimate after adjusting for age, sex and ejection fraction (**Table 5.4**). Moreover, this association persisted after further adjustment for severity of aortic stenosis (HR 1.77; 95%CI 1.22 to 2.35, P=0.002) as well as the coronary artery and aortic valve calcium scores (HR 2.10; 95%CI 1.22 to 3.61, P=0.007).

FIGURE 5.6. TEN-YEAR EVENT FREE SURVIVAL FOR COMPOSITE ENDPOINT FOR AORTIC VALVE REPLACEMENT OR CARDIOVASCULAR DEATHS



Patients in the highest tertile were associated with lower survival rates compared in the other tertiles (log rank test for trend, P=0.016)

TABLE 5.4. HAZARD RATIOS PREDICTING TIME TO VALVE REPLACEMENT OR CARDIOVASCULAR DEATH FOR TROPONIN I CONCENTRATIONS

Model	Hazard ratio (95% confidence interval)	P value
Model 1	1.65 (1.15 to 2.38)	0.007
Model 2	1.61 (1.11 to 2.35)	0.01
Model 3	1.63 (1.11 to 2.38)	0.01
Model 4	1.77 (1.22 to 2.55)	0.002
Model 5	2.10 (1.22 to 3.61)	0.007

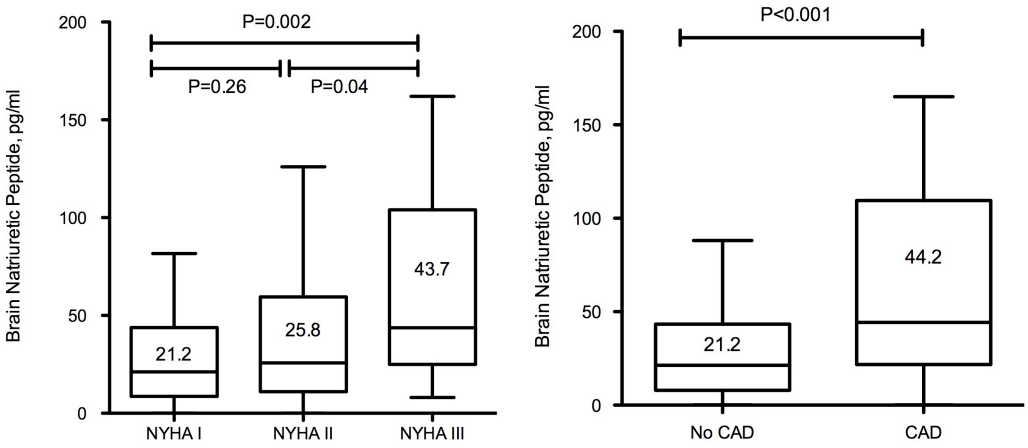
Model 1: Unadjusted
Model 2: Adjusting for age and sex
Model 3: Model 2 and systolic ejection fraction
Model 4: Model 2 and mean pressure gradient
Model 5: Model 2 and coronary and aortic valve calcium score

5.4.3 Mechanism and Prognosis Associated with BNP Concentrations

Serum BNP concentrations were higher in patients with aortic stenosis compared with healthy volunteers (26.4 [10.6,53.9] *versus* 10.3 [5.6,18.1] ng/ml, P=0.009; Table 1). In patients with aortic stenosis, BNP concentrations increased with age, disease severity, diastolic dysfunction, left ventricular mass index, myocardial fibrosis, the presence of coronary artery disease and symptoms (all p<0.05; **Figure 5.7**). However on multivariable analysis, only age was significantly associated with BNP concentrations (P<0.001; **Table 5.5**).

In the Outcome Cohort, NT-proBNP was not associated with aortic valve replacement or cardiovascular deaths in both unadjusted (HR 1.15 per two-fold increment in NT-proBNP concentration; 95%CI 0.86 to 1.53, P=0.34) and adjusted analyses (**Table 5.6**). Importantly, NT-proBNP concentration did not modify the association between troponin and time to aortic valve replacement or cardiovascular deaths (HR 1.60; 95% CI 1.10 to 2.34, P=0.01).

FIGURE 5.7. BRAIN NATRIURETIC PEPTIDE CONCENTRATIONS IN PATIENTS WITH AORTIC STENOSIS



In patients with aortic stenosis, brain natriuretic peptide (BNP) concentration was increased across NHYA functional class and in those with coronary artery disease (CAD).

Results were presented in box-and-whiskers plots (Tukey): the central box represents the interquartile range, with the median indicated by the line within the box. The whiskers extend to the most extreme values within the 1.5 interquartile ranges.

TABLE 5.5. UNIVARIATE AND MULTIVARIABLE ANALYSES TO EXAMINE THE DETERMINANTS OF BRAIN NATRIURETIC PEPTIDES CONCENTRATIONS IN PATIENTS WITH AORTIC STENOSIS

Variables	Univariate		Multivariable – Model 1 (Included %LGE)		Multivariable – Model 2 (Included ECV)	
	Relative change in BNP concentration (95% CI)	P value	Relative change in BNP concentration (95% CI)	P value	Relative change in BNP concentration (95% CI)	P value
Age, per 5 years	1.48 (1.35 to 1.62)	<0.001	1.95 (1.82 to 2.36)	<0.001	1.93 (1.58 to 2.34)	<0.001
Male sex	1.12 (0.63 to 1.99)	0.71				
Coronary artery disease	2.97 (1.75 to 5.10)	<0.001	1.42 (0.91 to 2.24)	0.17	1.86 (1.20 to 2.89)	0.01
Creatinine, per 10 μmol/l	1.17 (1.00 to 1.35)	0.05				
NYHA class	1.77 (1.26 to 2.48)	0.001	1.12 (0.84 to 1.49)	0.43	1.14 (0.85 to 1.54)	0.37
Mean pressure gradient, per 10 mmHg	1.22 (1.06 to 1.42)	0.007	1.08 (0.95 to 1.02)	0.25	1.09 (0.96 to 1.25)	0.16
Mean E/e'	1.08 (1.05 to 1.13)	<0.001	1.02 (0.99 to 1.05)	0.15	1.03 (1.00 to 1.70)	0.07
Ejection fraction, per 5%	0.95 (0.78 to 1.16)	0.60				
Indexed left ventricular mass, per 10 g/m^2	1.15 (1.02 to 1.28)	0.02	1.06 (0.95 to 1.02)	0.21	1.11 (1.01 to 1.25)	0.04
Extent of late gadolinium enhancement (%LGE), %	1.12 (1.04 to 1.19)	0.002	1.05 (0.99 to 1.12)	0.12		
Extracellular volume fraction, %	1.21 (1.08 to 1.39)	0.001			1.08 (0.97 to 1.18)	0.07

TABLE 5.6. HAZARD RATIOS FOR TIME TO VALVE REPLACEMENT OR CARDIOVASCULAR DEATH FOR N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE CONCENTRATIONS

Model	Hazard ratio (95% confidence interval)	P value
Model 1	1.15 (0.86 to 1.53)	0.34
Model 2	1.14 (0.80 to 1.60)	0.47
Model 3	1.13 (0.80 to 1.60)	0.49

Model 1: Unadjusted
Model 2: Adjusting for age and sex
Model 3: Model 2 and dyspnea (NYHA >1)

5.4.4 Exploratory Analysis of 1-year Change in Cardiac Troponin I Concentrations and Outcomes

Among a subset of patients in whom troponin was measured more than once and who were event-free at one year, we explored the association between both baseline troponin and change in troponin from baseline to year 1 with the odds of valve surgery or death during subsequent follow-up.

Serial plasma cardiac troponin I concentrations were available for 69 patients (52%). There was a strong correlation between baseline and one-year cTnI concentrations ($r=0.87$, $P<0.001$). Sixteen patients (23%) had an event within 3 years and 25 (41%) had an event within 5 years of follow-up. Associations in the same direction were evident for both 3-year and 5-year events for both baseline troponin (two fold increase OR 1.73 [1.11-2.89], P=0.02 and OR 1.39 [0.95-2.16], P=0.23, respectively) and difference in troponin from baseline (two fold increase OR 3.19 [1.33-8.62], P=0.01 and 1.58 [0.75-3.78], P=0.23, respectively), although only the associations for 3-year event rates were statistically significant.

5.5 DISCUSSION

This is the first dataset to explore mechanisms and outcomes associated with cTnI concentrations using a high-sensitivity assay in patients with aortic stenosis. In more than 250 patients with aortic stenosis, we have demonstrated that levels are detectable in 98% of subjects and increased compared to age and sex-matched healthy volunteers. Plasma cTnI concentrations were not associated with the presence of co-existent coronary artery disease or the severity of valve narrowing on multivariable analysis. Instead, plasma cTnI concentrations demonstrated a close association with the magnitude of left ventricular hypertrophy and the presence of mid-wall myocardial fibrosis. Moreover, high-sensitivity plasma cTnI concentration showed an independent association with long-term risk of aortic valve replacement or cardiovascular deaths. We therefore believe that high-sensitivity plasma cTnI concentrations hold potential as an objective marker of left ventricular decompensation in patients with aortic stenosis and as a potential early trigger to aortic valve replacement.

Aortic stenosis is defined not only by the development of progressive valve narrowing but also by the left ventricular hypertrophic response that ensues. Whilst this initially restores wall stress, decompensation due to progressive cell death and fibrosis ultimately occurs and patients transition from hypertrophy to heart failure (3). Because of the associated adverse prognosis, current guidelines recommend surgery in patients with severe stenosis and evidence of such decompensation, detected either on the basis of symptom development or an ejection fraction <50%. Unfortunately, symptoms are often frequently difficult to assess whilst an ejection fraction <50% occurs late in the disease process and is often irreversible. There is therefore emerging interest in developing novel, objective biomarkers of decompensation for patients with aortic stenosis. Data from our study suggests that troponin has the potential to be such a marker.

To date elevated cardiac troponin has been considered the *sine qua non* for the diagnosis of myocardial infarction (170). However, marked improvements in

assay sensitivity now allow quantification of plasma cTnI concentrations in the majority of the healthy population (116). In our study, cTnI was detectable in 98% of patients with aortic stenosis, and exceeded the recommended diagnostic threshold for myocardial infarction in 7.9%. Patients with stable coronary disease have been reported to have higher plasma troponin concentrations, with elevated levels being associated with long-term cardiovascular risk (171). However, in our cohort of patients with aortic stenosis, there were no differences in plasma troponin concentrations between those with and without coronary artery disease. Instead, plasma troponin concentrations were independently associated with an advanced hypertrophic response and replacement myocardial fibrosis. Indeed, the latter occurred over and above the effects of left ventricular mass, supporting our hypothesis that cTnI release relates to the myocardial injury that accompanies ventricular decompensation and myocardial fibrosis.

The poor prognosis associated with increased troponin concentrations offers further support for this model. At ten years, more than a half of patients in the highest tertile of plasma cTnI had undergone an aortic valve replacement or died from cardiovascular disease. Moreover, plasma cTnI concentrations were associated with aortic valve replacement or cardiovascular deaths, independent of the burden of coronary atherosclerosis (as assessed using coronary calcium scoring) as well as age, sex, systolic ejection fraction, echocardiographic measures of aortic stenosis severity and the aortic valve calcium score.

A recent study demonstrated an association between high-sensitivity cardiac troponin T concentrations and echocardiographic measures of left ventricular modeling in aortic stenosis (117). Our data confirms and extends these findings using cardiovascular magnetic resonance, which has allowed us to investigate the remodeling response in greater detail and crucially assess the relationship with myocardial fibrosis, thereby providing additional mechanistic data. We therefore believe that the plasma cTnI concentration measured by a high-sensitivity assay has considerable potential as an early biomarker of left ventricular decompensation and as a powerful prognostic

tool in patients with aortic stenosis. Moreover, this test is inexpensive and easy to perform making any future transition into routine clinical practice readily achievable. However, considerable overlap was observed between patients with aortic stenosis and our control cohort. This is perhaps unsurprising given cTnI is released as a consequence of a wide range of myocardial insults. A future strategy where asymptomatic aortic stenosis patients with elevated or increasing plasma troponin concentrations subsequently proceed to cardiovascular magnetic resonance for confirmation of myocardial fibrosis and left ventricular decompensation is therefore attractive. Large-scale prospective studies are now required to investigate the use of these two biomarkers in the management and risk stratification of patients with aortic stenosis and whether the above approach might identify asymptomatic patients who would benefit from early surgery.

In contrast to troponin, BNP did not have prognostic value in our study. BNP is an endogenous cardiac hormone released in response to increasing left ventricular wall stress and most commonly used in the assessment of patients with heart failure. It is therefore only likely to be released late in the transition from hypertrophy to heart failure, making it of limited value in detecting signs of early decompensation in asymptomatic patients. Given that this is the group in whom novel biomarkers of left ventricular decompensation are most likely to be useful, we believe that troponin holds greater clinical promise than BNP.

5.5.1 Study Limitations

Cardiovascular magnetic resonance was not available at the inception of the SALTIRE study. Therefore, we needed to recruit another patient population to investigate the mechanism for troponin release in aortic stenosis. However, plasma cTnI concentrations in the Outcome Cohort also displayed a close association with left ventricular mass determined by echocardiography, and were unrelated to the burden of coronary atherosclerosis or the severity of valvular stenosis. Similar mechanisms would therefore seem to govern cTnI release across both groups. Another limitation is the lack of more sensitive markers of left ventricular systolic dysfunction in the Mechanism Cohort, for example cardiovascular magnetic resonance tagging techniques. However, we elected not to perform these due to concerns about lengthening the scanning protocol in this elderly cohort of patients and compromising the detection of myocardial fibrosis. Finally, data on short-term biological variability (the change in concentration from one occasion to another) is very limited in disease states. However, we do not anticipate significant short-term variability in chronic conditions such as aortic stenosis, although this will require further validation.

5.6 CONCLUSIONS

In patients with aortic stenosis, plasma cTnI concentrations are a marker of left ventricular decompensation and myocardial fibrosis that are associated with cardiovascular deaths and aortic valve replacement. High-sensitivity troponin assays hold major promise as a future clinical tool for patients with this condition.

CHAPTER 6

LEFT VENTRICULAR HYPERTROPHY WITH STRAIN AND AORTIC STENOSIS

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Shah AS*, **Chin CW***, Vassiliou V, Cowell SJ, Doris M, Kwok TC, Semple S, Zamvar V, White AC, McKillop G, Boon NA, Prasad SK, Mills NL, Newby DE, Dweck MR. Left ventricular hypertrophy with strain and aortic stenosis. **Circulation** 2014;130:1607-1616.

* Equal contributions as first authors

6.1 SUMMARY

AIMS: Electrocardiographic (ECG) left ventricular hypertrophy (LVH) with strain is associated with an adverse prognosis in aortic stenosis. We investigated the mechanisms and outcomes associated with ECG-strain.

METHODS: One hundred and two patients (70 [63,75] years, 66% males, aortic valve area 0.9 [0.7,1.2] cm^2) underwent ECG, echocardiography and cardiovascular magnetic resonance: the Mechanism Cohort. Myocardial fibrosis was determined using late gadolinium enhancement (LGE, replacement fibrosis) and T1 mapping (diffuse fibrosis). The relationship between ECG-strain and CMR was then assessed in an external Validation Cohort (n=64). The Outcome Cohort comprised of 140 patients from the Scottish Aortic Stenosis and Lipid Lowering Trial Impact on REgression (SALTIRE) study and followed up for 10.6 years (1,254 patient-years).

RESULTS: Compared to those without LVH (n=51) and LVH without ECG-strain (n=30), patients with ECG-strain (n=21) had more severe aortic stenosis, increased left ventricular mass index, more myocardial injury (high-sensitivity plasma cardiac troponin I concentration 4.3 [2.5,7.3] *versus* 7.3 [3.2,20.8] *versus* 18.6 [9.0,45.2] ng/L respectively, P<0.001) and increased diffuse fibrosis (extracellular volume fraction 27.4±2.2 *versus* 27.2±2.9 *versus* 30.9±1.9% respectively, P<0.001). All patients with ECG-strain had mid-wall LGE (positive and negative predictive values of 100% and 86%, respectively). Indeed, LGE was independently associated with ECG-strain (OR 1.73, 95%CI 1.08-2.77, P=0.02): a finding confirmed in the Validation Cohort. In the Outcome Cohort, ECG-strain was an independent predictor of aortic valve replacement or cardiovascular death (HR 2.67, 95%CI 1.35-5.27, P<0.01).

CONCLUSIONS: ECG-strain is a specific marker of mid-wall myocardial fibrosis and predicts adverse clinical outcomes in aortic stenosis.

6.2 INTRODUCTION

Aortic stenosis is characterized by progressive valve narrowing and secondary changes in the myocardium (3). In response to increased afterload, left ventricular hypertrophy can develop in order to maintain wall stress and cardiac function. Although this process appears to be compensatory in the early stages, pre-clinical studies have suggested cardiac performance can be preserved in the absence of hypertrophy (172,173). Moreover, the left ventricular hypertrophic response ultimately decompensates with progressive cell death and fibrosis driving the transition to symptoms, heart failure and adverse cardiovascular events (3,36,95). There is therefore considerable interest in identifying early, objective markers of this decompensation that might identify asymptomatic patients who would benefit from early valve replacement.

Electrocardiographic (ECG) strain is a well-recognized marker of left ventricular hypertrophy. However, the exact mechanism underlying the characteristic ST and T wave abnormalities associated with this pattern is uncertain. In this study, we hypothesized that ECG-strain is a marker of left ventricular decompensation, and investigated this association using cardiovascular magnetic resonance to assess the degree of left ventricular hypertrophy and myocardial fibrosis, and high-sensitivity plasma cardiac troponin I (cTnI) as a marker of myocardial injury. Moreover, we aimed to reassess the adverse prognosis previously associated with the ECG-strain pattern in patients with aortic stenosis (8).

6.3 METHODS

Three cohorts were used for the study. In the Mechanism Cohort, we determined the pathophysiology underlying the ECG-strain pattern using cardiovascular magnetic resonance and plasma cTnI concentration in patients recruited from the Edinburgh Heart Centre. This was then independently validated in an external Validation Cohort from the Royal Brompton Hospital, London. Subsequently in the Outcome Cohort, we examined the prognostic role of ECG-strain in patients with aortic stenosis. The study was conducted in accordance with the Declaration of Helsinki, and approved by the local research ethics committee. Written informed consent was obtained from all participants.

6.3.1 Patient Populations

Mechanism Cohort: Patients with aortic stenosis (mild to severe) were recruited prospectively from the Edinburgh Heart Centre. In addition to the exclusion criteria stated in Chapter 2, we also excluded patients with left or right bundle branch block, concurrent digoxin use, and impaired systolic function on cardiovascular magnetic resonance (ejection fraction <95th centile for age and sex) (129).

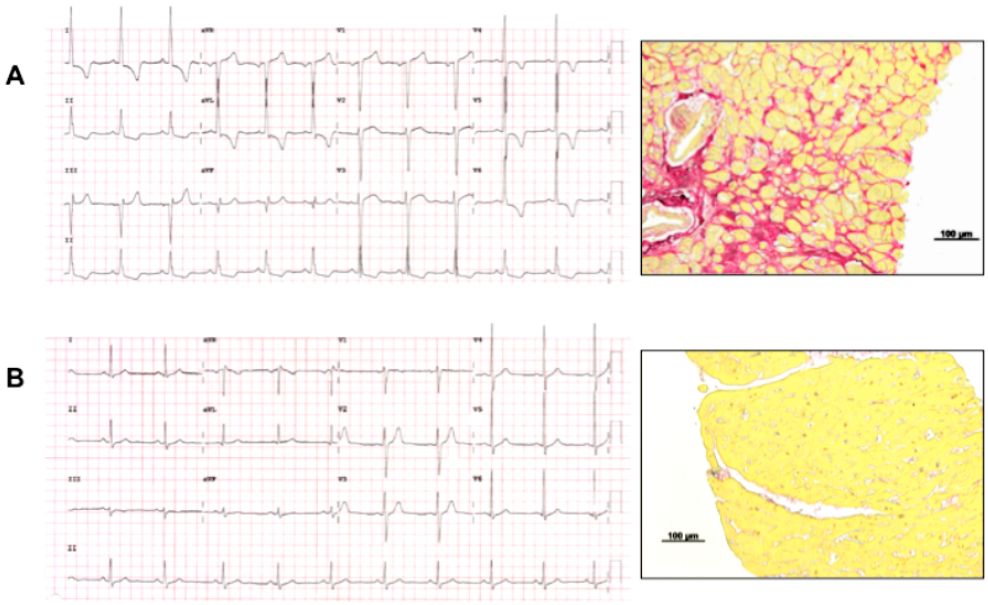
Validation Cohort: Between 2011 and 2013, patients with moderate to severe aortic stenosis undergoing CMR were prospectively recruited from the Royal Brompton Hospital, London, using similar exclusion criteria.

Outcome Cohort: Patients were initially recruited into the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on REgression (SALTIRE) study between March 2001 and April 2002, which comprised 155 patients with asymptomatic aortic stenosis who had been randomly assigned to either atorvastatin 80 mg or placebo once daily. Patients were excluded if already on a statin, or if aortic valve replacement was planned (due to either symptoms or impaired systolic function) (127). For the purposes of this analysis, patients on digoxin or with uninterpretable ECGs, or bundle branch block patterns were excluded.

6.3.2 Electrocardiography

A standard 12-lead ECG was obtained in all participants and interpretation of the ECG was performed independently by two observers who were blinded to the clinical data and imaging findings. Left ventricular hypertrophy on ECG was diagnosed based on the Romhilt-Estes point system (≥5 points) (125) and ECG-strain was defined as ≥1 mm concave downsloping ST depression with asymmetrical T wave inversion in the lateral leads (**Figure 6.1A**) (124).

FIGURE 6.1. ELECTROCARDIOGRAMS AND MYOCARDIAL BIOPSIES IN TWO PATIENTS WITH SEVERE AORTIC STENOSIS



The electrocardiogram for patient A (**A**) demonstrated left ventricular hypertrophy and associated repolarization abnormalities (ST segment depression and asymmetrical T wave inversion in the lateral leads) characteristic of the electrocardiographic strain pattern, whereas the electrocardiogram for patient B (**B**) demonstrated left ventricular hypertrophy without the strain pattern. Compared with patient B, patient A had increased left ventricular mass index (169 *versus* 81 g/m^2), increased plasma cardiac troponin I concentrations (84 *versus* 2.5 ng/L), and evidence of more extensive myocardial fibrosis on both cardiovascular magnetic resonance and histology (picrosirius red staining).

6.3.3 Imaging Protocols

Transthoracic echocardiography was performed in all participants in the Mechanism and Outcome Cohorts according to the protocol described in Chapter 2. Cardiovascular magnetic resonance in the Mechanism Cohort was performed at 3T and the methodology has been described in detail in Chapter 2. In addition, we also assessed left ventricular longitudinal shortening on cardiovascular magnetic resonance by measuring the difference in mitral annular displacement between end-systole and end-diastole. The mean value of the lateral and septal insertion sites (4-chamber view) and the anterior and inferior sites (2-chamber view) was used. In the Validation Cohort, cardiovascular magnetic resonance was performed at 1.5T, as previously described (58).

6.3.4 High-Sensitivity Plasma Cardiac Troponin I Assay

Plasma cTnI concentrations were determined in patients in the Mechanism Cohort as a marker of myocyte injury using the ARCHITECT *STAT* high-sensitive troponin I assay (Abbott Laboratories, Abbott Park, Illinois). The method of detection and sensitivity of the assay have been described in Chapter 2. Concentrations lower than the detection limit were assigned a value of 1.2 ng/L.

6.3.5 Calcium Scoring in the Outcome Cohort

ECG-gated non-contrast computed tomography scans of the coronary arteries and aortic valve were performed in all patients in the Outcome Cohort using a double helix scanner (Twin II Flash, Philips Medical Systems). Coronary artery and aortic valve calcium scores were determined by a single operator using the Picker Cardiac Scoring software (127).

6.3.6 Long-term Follow-up in the Outcome Cohort

Clinical outcomes were obtained in the Outcome Cohort and adjudicated by two independent investigators blinded to the clinical and electrocardiographic data. In-hospital and community deaths were captured from the General Register of Scotland. Cardiovascular death was defined as death due to myocardial infarction, sudden cardiac death, heart failure, stroke, death related to cardiovascular procedures, and death due to other cardiovascular causes. Each death was classified by the two independent investigators and any discrepancy was resolved by consensus. Furthermore, all events, including surgical aortic valve replacements (no patients had transcatheter aortic valve implantation during follow-up), were confirmed by independent review of each patient's healthcare record. All patients in the Outcome Cohort were managed in our tertiary cardiac centre, and reviewed at a multi-disciplinary meeting prior to undergoing aortic valve replacement. Only patients with established indications as per contemporary guidelines were referred for aortic valve replacement (5,169).

6.3.7 Statistical Analysis

In the Mechanism Cohort, the association between ECG-strain and left ventricular mass and aortic stenosis severity was assessed using multivariable linear regression analysis to adjust for potential confounders. Furthermore, we assessed determinants associated with ECG-strain using univariate and multivariable logistic regression.

In the Outcome Cohort, time-to-event curves in patients with and without ECG-strain were estimated using the Kaplan-Meier method and compared using the log-rank test. To accommodate for competing risks, the association between time to aortic valve replacement or cardiovascular death and ECG-strain was modeled as a composite outcome in Cox proportional hazards models. The assumption for proportional hazards was assessed using the log (-log [survival]) *versus* log (survival time) plots and by examining the Schoenfeld residuals using R version 2.15.2 (Vienna, Austria). Survival analyses and other standard statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA) and SPSS Version 19 (SPSS, Inc., Chicago, IL., USA), respectively.

6.4 RESULTS

One hundred and two patients with aortic stenosis (70 [63, 75] years, 66% males, aortic valve area 0.9 [0.7, 1.2] cm^2) were recruited into the Mechanism Cohort with a further 64 patients recruited into the Validation Cohort (79 [69, 84] years, 69% males, aortic valve area 0.9 [0.7, 1.0] cm^2) (**Tables 6.1 and 6.2**). After excluding patients with uninterpretable ECGs, bundle branch block or receiving digoxin therapy (n=15), 140 patients from the SALTIRE study were analyzed as part of the Outcome Cohort (69 [62, 75] years, 70% males, aortic valve area 1.0 [0.7, 1.3] cm^2) (**Table 6.3**). All patients in the Mechanism and Outcome Cohorts were Caucasians. In the Validation Cohort, 92% were Caucasians and the remainder South Asians. There were no observed racial differences with respect to the presence of left ventricular hypertrophy or strain on the ECG (P=0.95; **Table 6.2**).

TABLE 6.1. BASELINE CHARACTERISTICS OF PATIENTS IN THE MECHANISM COHORT

	All patients (n=102)	Patients with aortic stenosis			
		No LVH (n=51)	LVH without strain (n=30)	ECG Strain (n=21)	P value
CLINICAL CHARACTERISTICS					
Age, years	70 [63.75]	70 [65,75]	70 [65,73]	69 [61,75]	0.83
Sex, males, n (%)	67 (66)	30 (59)	23 (77)	16 (76)	0.09
Diabetes mellitus, n (%)	8 (8)	5 (10)	2 (7)	2 (10)	0.88
Hypertension, n %	61 (60)	29 (58)	21 (70)	11 (52)	0.99
Coronary artery disease, n (%)	28 (27)	11 (22)	9 (30)	8 (38)	0.14
Systolic blood pressure, mmHg	146±21	146±22	146±20	147±20	0.98
Bicuspid aortic valve, n (%)	33 (32)	13 (25)	12 (40)	8 (38)	0.21
NYHA Class, n (%) III and IV	22 (22)	4 (8)	6 (20)	12 (57)	<0.001
ECHOCARDIOGRAPHY					
Aortic valve area, cm^2	0.9 [0.7,1.2]	1.0 [0.7,1.3]	0.9 [0.7,1.1]	0.7 [0.6,0.9]	0.02
Aortic jet velocity, m/s	3.8±1.0	3.2±0.7	3.8±0.8	4.8±1.1	<0.001[a,b,c]
Mean pressure gradient, mmHg	30 [20,39]	23 [14,32]	31 [22,41]	45 [37,64]	<0.001
Dimensionless index	0.29±0.10	0.31±0.10	0.28±0.09	0.25±0.09	0.03[c]
Mid-wall fractional shortening, mm	10.9±1.7	11.2±1.9	10.9±1.5	9.9±1.2	0.01[c]
Mitral E/A ratio	1.0±0.4	0.9±0.4	1.0±0.4	0.9±0.4	0.51
Deceleration time, ms	210±56	197±51	214±57	235±57	0.02
Mean e', cm/s	6.2±1.9	6.7±2.0	6.3±1.7	4.9±1.5	<0.01[c]
E/e' ratio	12.5 [10.0,16.7]	11.7 [9.4,14.9]	12.3 [9.3,15.4]	17.0 [13.0,23.0]	<0.001[b,c]
CARDIOVASCULAR MAGNETIC RESONANCE					
Indexed left ventricular mass, g/m^2	91±24	75±14	99±18	118±22	<0.001[a,b,c]
Indexed end-diastolic volume (EDVi), ml/m^2	69 [62,78]	67 [60,71]	77 [69,88]	73 [65,86]	<0.001
Indexed end-systolic volume, ml/m^2	22 [18.27]	22 [17,25]	25 [21,29]	23 [19,29]	0.03
Indexed stroke volume, ml	49±10	45±8	51±10	54±12	<0.01[a,c]
LVMi/EDVi, g/mL	1.27±0.26	1.14±0.22	1.33±0.21	1.51±0.22	<0.001[a,b,c]
Ejection fraction, %	68±6	68±5	67±6	68±8	0.90
Longitudinal shortening, mm	12.4±3.1	13.1±2.7	12.9±3.1	9.9±2.7	<0.001[b,c]
Patients with mid-wall late gadolinium enhancement, n (%)	32 (31)	4 (8)	7 (23)	21 (100)	<0.001
Amount of late gadolinium enhancement, %	0 [0,5.5]	3.9 [1.8,7.0]	5.8 [5.0,7.6]	9.5 [7.5,14.2]	<0.01
Extracellular volume fraction, %	28.1±2.8	27.4±2.2	27.2±2.9	30.9±1.9	<0.001[b,c]
PLASMA CARDIAC TROPONIN I CONCENTRATION					
Cardiac troponin I concentrations, ng/L	6.7 [3.6,13.3]	4.3 [2.5, 7.3]	7.3 [3.2, 20.8]	18.6 [9.0, 45.2]	<0.001

ANOVA post-hoc Bonferroni adjustment: [a]P<0.05 between no LVH and LVH without strain; [b]P<0.05 between LVH without strain and LVH with strain; [c]P<0.05 between no LVH and LVH with strain.

TABLE 6.2. BASELINE CHARACTERISTICS OF PATIENTS IN THE EXTERNAL VALIDATION COHORT

	All patients (n=64)	Patients with aortic stenosis			P value
		No LVH (n=48)	LVH without strain (n=5)	ECG Strain (n=11)	
CLINICAL CHARACTERISTICS					
Age, years	76 [69,84]	78 [68,83]	80 [63,89]	80 [73,87]	0.34
Sex, males, n (%)	44 (69)	32 (67)	4 (80)	8 (73)	0.26
Diabetes mellitus, n (%)	16 (25)	13(27)	0	3 (27)	0.78
Hypertension, n %	33 (52)	26(54)	1 (20)	6 (55)	0.76
Coronary artery disease, n (%)	26 (41)	21(44)	1 (20)	4 (36)	0.51
Systolic blood pressure, mmHg	133 [119,142]	134 [121,142]	132 [124.158]	123 [110.140]	0.49
Bicuspid aortic valve, n (%)	14(22)	11(23)	0	3 (27)	0.97
NYHA Class, n (%) III and IV	14 (22)	11(23)	0	3 (27)	0.97
Race					
Caucasians	59 (92)	44 (92)	5 (100)	10 (91)	0.95
South Asians	5 (8)	4 (8)	0	1 (9)	
CARDIOVASCULAR MAGNETIC RESONANCE					
Planimetered aortic valve area, cm^2	0.9 [0.7,1.0]	0.8 [0.7, 1.0]	1.0 [0.7,1.3]	0.7 [0.7,0.9]	0.12
Indexed left ventricular mass (LVMi), g/m^2	88 [74,113]	85 [72,107]	82 [74,113]	121 [102,133]	0.02
Indexed end-diastolic volume (EDVi), mL/m^2	76 [67,104]	74 [64,95]	86 [67,97]	120 [75,153]	0.01
Indexed end-diastolic volume, mL/m^2	28 [17,48]	27 [17,42]	29 [17,36]	65 [38,102]	<0.01
Indexed stroke volume, mL	48 [38,55]	47 [40,54]	55 [47,65]	49 [36,52]	0.19
LVMi/EDVi, g/mL	1.17 [0.86,1.38]	1.18 [0.91,1.35]	1.05 [0.88,1.42]	1.13 [0.79,1.48]	0.92
Ejection fraction, %	64[46,72]	65 [51,73]	68 [63,76]	44 [32,48]	<0.01
Patients with mid-wall LGE, n (%)	25 (39)	12 (25)	3 (60)	10 (91)‡	0.02

‡The remaining patient had a large infarct

TABLE 6.3. CHARACTERISTICS OF PATIENTS IN THE OUTCOME COHORT

		Patients with aortic stenosis		
	All patients (n=140)	No ECG-strain (n=120)	ECG-strain (n=20)	P value
CLINICAL CHARACTERISTICS				
Age, years	69 [62, 75]	69 [61, 75]	75 [66, 77]	0.05
Sex, males, n (%)	98 (70)	82 (68)	16 (80)	0.43
Diabetes mellitus, n (%)	4 (3)	4 (3)	0	0.59
Hypertension, n (%)	71 (50)	58 (48)	13 (65)	0.23
Coronary artery disease, n (%)	24 (17)	20 (17)	4 (20)	0.75
Systolic blood pressure, mmHg	144±19	144±20	142±16	0.65
ECHOCARDIOGRAPHY				
Aortic valve area, cm^2	1.0 [0.7,1.3]	1.0 [0.7,1.3]	0.6 [0.4,0.8]	0.03
Peak aortic jet velocity, m/s	3.4 [2.8,4.0]	3.2 [2.8,3.9]	3.9 [3.5,4.4]	<0.01
Mean pressure gradient, mmHg	24 [18,35]	22 [17,33]	34 [26,44]	<0.001
Ejection Fraction, %	69±10	70±11	69±9	0.70
Indexed left ventricular mass, g/m^2	173 [142, 205]	164 [131, 200]	203 [177, 223]	<0.01
COMPUTED TOMOGRAPHY				
Coronary calcium score, log AU	1.6±1.2	1.6±1.3	1.7±1.2	0.69
Aortic valve calcium score, log AU	3.6±0.6	3.6±0.6	4.0±0.4	0.01
PLASMA CARDIAC TROPONIN I CONCENTRATION				
Cardiac troponin I concentrations, ng/L	7.5 [5.7,13.4]	6.9 [5.3,11.4]	17.3 [10.5,29.6]	<0.001

6.4.1 Mechanisms Underlying ECG-strain

Fifty-one patients in the Mechanism Cohort fulfilled ECG criteria for left ventricular hypertrophy, demonstrating high predictive values for the presence of left ventricular hypertrophy on cardiovascular magnetic resonance (positive predictive value 96%, negative predictive value 89%). Of these, 21 patients had the strain pattern on their ECGs. These patients with ECG-strain had the highest left ventricular mass index and most severe aortic stenosis compared to other patient groups (those without left ventricular hypertrophy on their ECG and those with left ventricular hypertrophy but no ECG-strain) (**Table 6.1**), even after the adjustment for age, sex and systolic blood pressure (P<0.001 for both). Moreover, compared to other groups, these patients had increased end-diastolic volumes (P<0.01), worse diastolic function (P<0.001) and more severe symptoms (P<0.001; **Table 6.1**). Despite similar left ventricular ejection fraction, patients with left ventricular hypertrophy and ECG-strain also had the worst longitudinal function, diastolic function (**Figure 6.2**) and mid-wall fractional shortening (**Table 6.1**).

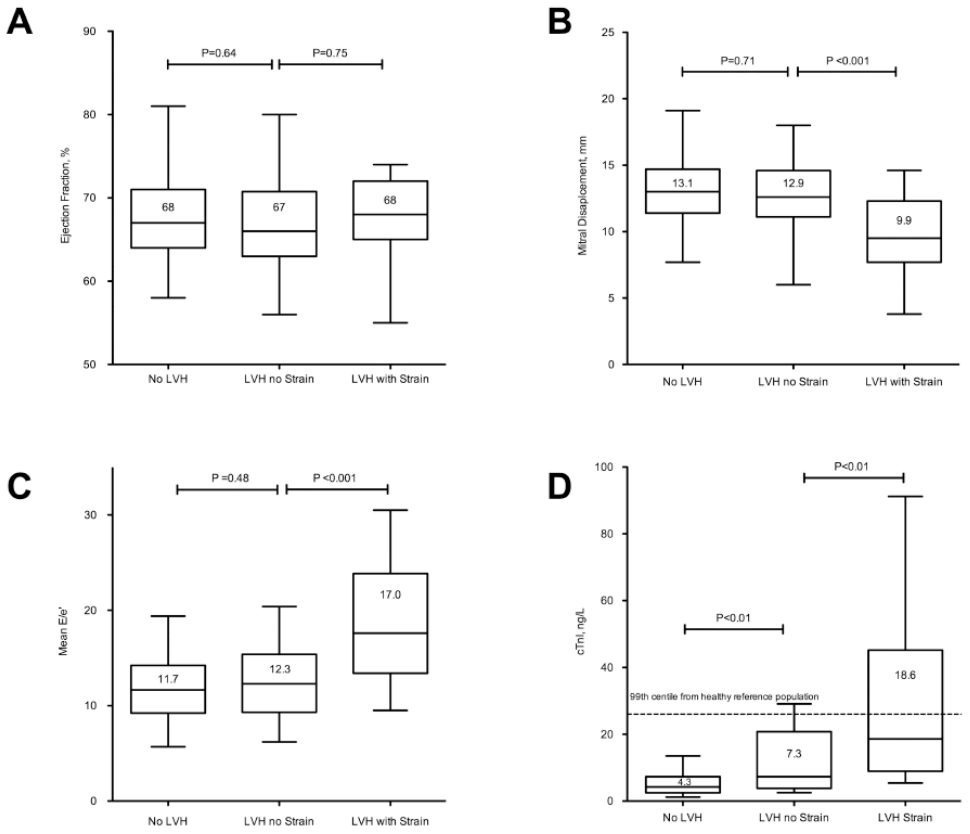
Interestingly, all patients with left ventricular hypertrophy and ECG-strain had focal mid-wall fibrosis (positive and negative predictive value of 100% and 86%, respectively; **Figure 6.3B**), strongly supporting ECG-strain as a specific marker of replacement myocardial fibrosis. Moreover these patients had more extensive diffuse myocardial fibrosis (Extracellular volume fraction: 30.9±1.9 *versus* 27.2±2.9 in patients with left ventricular hypertrophy and no ECG strain *versus* 27.4±2.2% in patients without left ventricular hypertrophy, P<0.001; **Figure 6.3A**) and myocardial injury as assessed by high-sensitivity plasma cTnI. Indeed, plasma cTnI concentrations were more than 4-fold higher in patients with strain than in the other patient groups (18.6 [9.0, 45.2] *versus* 7.3 [3.2, 20.8] ng/L in patients with left ventricular hypertrophy and no ECG-strain *versus* 4.3 [2.5, 7.3] ng/L in patients without left ventricular hypertrophy, P<0.001; **Figure 6.2D**). Three patients with ECG-strain had both an infarct and mid-wall pattern of fibrosis on late gadolinium enhancement, and our findings remained unchanged even after their exclusion.

On univariate analysis, ECG-strain was associated with an increased left ventricular mass index, more severe aortic stenosis, increased replacement and diffuse myocardial fibrosis, and diastolic dysfunction (all P<0.01; **Table 6.4**) but was not associated with the presence of coronary artery disease (OR 1.88; 95% confidence interval 0.68 to 5.18; P=0.22). However, only increased myocardial fibrosis (either amount of late gadolinium enhancement or extracellular volume fraction) and the severity of aortic stenosis maintained an independent association on multivariate analysis with increased left ventricular mass index, increased myocardial injury and diastolic dysfunction all dropping out of the model (Models 3 and 4 in **Table 6.4**).

Myocardial histology was available in two patients who underwent aortic valve replacement and biopsy, supporting increased myocardial fibrosis in patients with left ventricular hypertrophy and ECG-strain (**Figure 6.1**). However, not all patients with myocardial late gadolinium enhancement had a strain pattern on the ECG. Indeed, of the 32 patients with myocardial late gadolinium enhancement, 11 patients (34%) did not have any evidence of ECG repolarization abnormalities. Interestingly, these patients had ~40% less replacement fibrosis on late gadolinium enhancement compared to patients who had ECG-strain (5.6 [4.3, 7.5] *versus* 9.5 [7.5, 14.2] %, P=0.002) with no differences in the distribution of mid-wall late gadolinium enhancement between these groups (P=0.78).

In the external Validation Cohort, similar findings were demonstrated (**Table 6.2; Figure 6.4**). There were 11 patients with ECG-strain, of whom 10 had isolated mid-wall fibrosis and one had extensive fibrosis from a large myocardial infarct to explain the ECG changes. Conversely, 15 patients had mid-wall fibrosis but no ECG-strain. In this cohort of patients with moderate to severe aortic stenosis, the positive and negative predictive values of left ventricular hypertrophy with ECG-strain for mid-wall fibrosis were 91% and 72%, respectively. Patients with ECG-strain were again observed to have an advanced hypertrophic response associated with increased left ventricular mass index and reduced myocardial performance (**Table 6.2**).

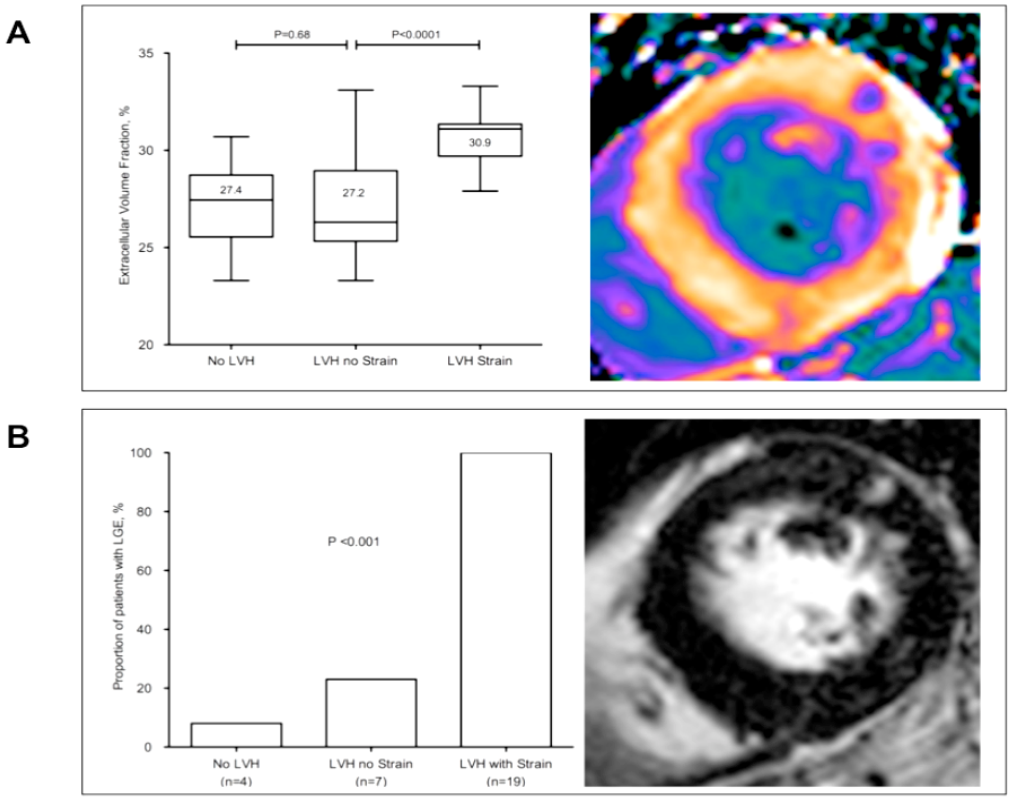
FIGURE 6.2. MYOCARDIAL PERFORMANCE AND CARDIAC TROPONIN I CONCENTRATIONS IN PATIENTS WITH ELECTROCARDIOGRAPHIC STRAIN PATTERN



Despite similar normal-range ejection fractions (**A**), patients with left ventricular hypertrophy and electrocardiographic strain had the most impaired longitudinal shortening (**B**) and diastolic function (**C**). High sensitivity plasma cardiac troponin I concentrations were four-fold higher in patients with electrocardiographic strain compared with patients without left ventricular hypertrophy on electrocardiogram (**D**).

Results were presented in box-and-whiskers plots (Tukey): the central box represents the interquartile range, with the median indicated by the line within the box. The whiskers extend to the most extreme values within the 1.5 interquartile ranges.

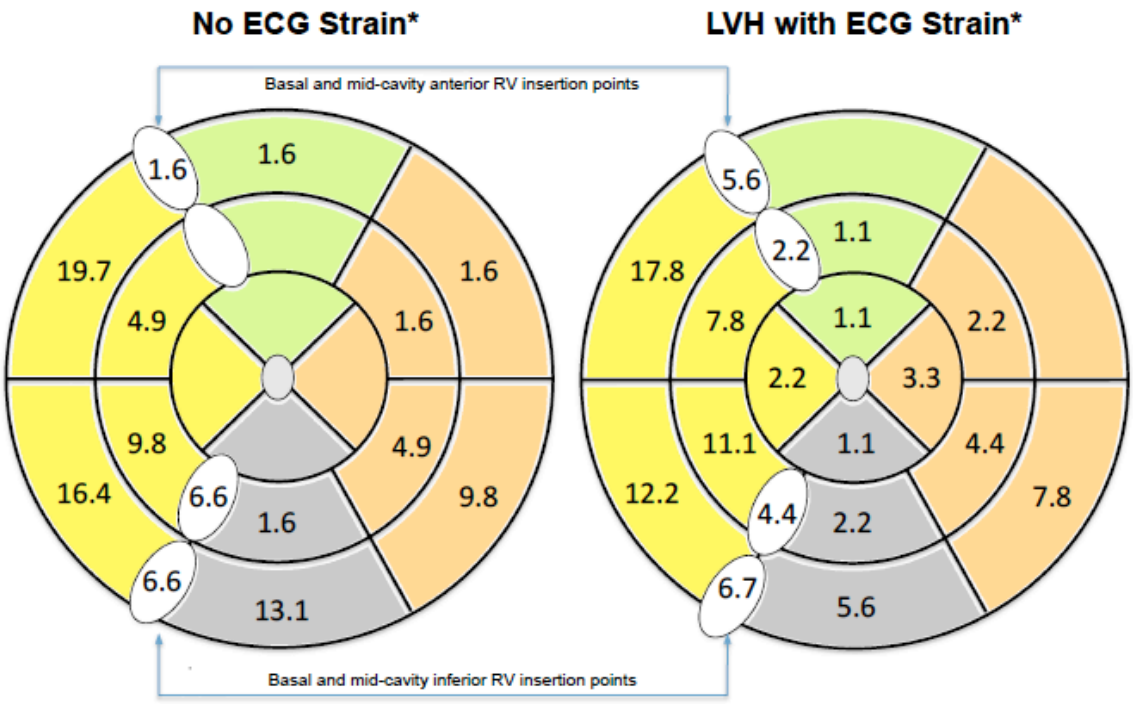
FIGURE 6.3. ASSOCIATION BETWEEN MYOCARDIAL FIBROSIS AND PATIENTS WITH ELECTROCARDIOGRAPHIC STRAIN PATTERN



Patients with strain pattern on the electrocardiogram had increased extracellular volume fractions, suggestive of increased diffuse myocardial fibrosis (**A**). Furthermore, all patients with electrocardiographic strain had a mid-wall pattern of late gadolinium enhancement (**B**). Of note, about a third of patients with mid-wall late gadolinium enhancement did not have electrocardiographic strain. The corresponding myocardial T1 map (**A**) and late gadolinium enhancement (**B**) of a patient with electrocardiographic strain demonstrated evidence of focal myocardial fibrosis in the mid-cavity lateral wall. The extracellular volume fraction of the mid-cavity slice in this patient was 30.2%.

Results in (**A**) presented in box-and-whiskers plots (Tukey): the central box represents the interquartile range, with the median indicated by the line within the box. The whiskers extend to the most extreme values within the 1.5 interquartile ranges.

FIGURE 6.4. DISTRIBUTION OF MID-WALL FIBROSIS



** Proportion of segments with mid-wall myocardial fibrosis (%)*

Mid-wall fibrosis was predominantly found in the basal and mid-cavity (92% and 100% of all segments with late gadolinium enhancement in patients with and without ECG strain, respectively). Whilst late gadolinium enhancement was observed more commonly in the septum, inferior and inferolateral than anterior segments, the distribution was not different between those with and without ECG strain (P=0.78).

TABLE 6.4. UNIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION ANALYSES TO ASSESS DETERMINANTS OF ELECTROCARDIOGRAPHIC STRAIN PATTERN

	Univariate Analysis		Multivariate Analysis (Model 1)		Multivariate Analysis (Model 2)		Multivariate Analysis (Model 3)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age, per 10 years	0.91 (0.61 to 1.36)	0.56	0.88 (0.38 to 2.03)	0.76	-	-	-	-
Male sex	1.69 (0.56 to 5.10)	0.35	0.54 (0.07 to 3.93)	0.54	-	-	-	-
Coronary artery disease	1.88 (0.68 to 5.18)	0.22	-	-	-	-	-	-
Mean pressure gradient, per 10 mmHg	1.80 (1.31 to 2.48)	<0.001	1.88 (1.02 to 1.13)	<0.01	1.93 (1.04 to 3.60)	0.03	2.10 (1.22 to 3.60)	0.01
Indexed left ventricular mass, per 10 g/m^2	2.10 (1.49 to 2.95)	<0.001	1.95 (1.14 to 3.35)	<0.01	1.30 (0.63 to 2.66)	0.47	1.77 (0.97 to 3.22)	0.06
Amount of late gadolinium enhancement, %	1.75 (1.35 to 2.27)	<0.001	-	-	1.73 (1.08 to 2.77)	0.02	-	-
Extracellular volume fraction, %	1.86 (1.38 to 2.47)	<0.001	-	-	-	-	1.55 (1.04 to 2.31)	0.03
Cardiac troponin I concentrations, ng/L‡	3.14 (1.73 to 5.71)	<0.001	3.30 (1.24 to 8.80)	0.02	3.18 (0.62 to 16.26)	0.16	2.43 (0.83 to 7.10)	0.11
Mean e', cm/s	0.51 (0.34 to 0.75)	<0.01	-	-	1.71 (0.38 to 7.54)	0.71	0.95 (0.46 to 1.94)	0.88

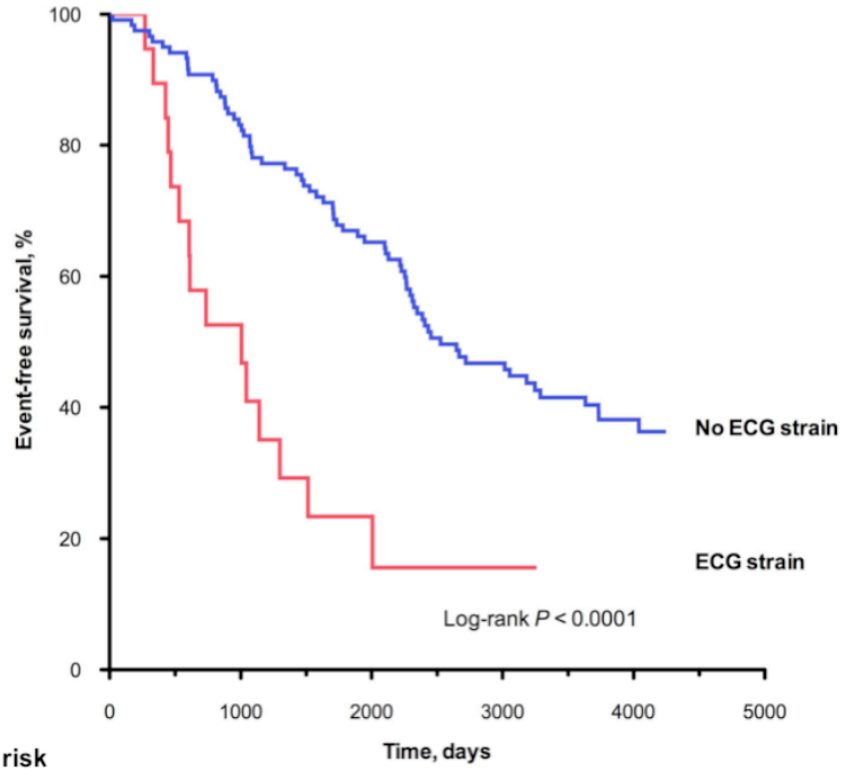
‡Log-transformed

6.4.2 Prognostic Value of ECG Strain

In the Outcome Cohort, 20 patients (14%) had left ventricular with strain on ECG. Consistent with the Mechanism Cohort, patients with ECG-strain had more severe aortic stenosis, increased left ventricular mass index and elevated plasma cTnI concentrations compared to those without strain (**Table 6.3**). Of note, these elevated cTnI concentrations in patients with ECG-strain were similar to those observed in the Mechanism Cohort (P=0.85).

Over 10.6 years of follow-up (1,254 patient-years), 63 patients had an aortic valve replacement and 22 patients died from a cardiovascular cause out of a total of 36 deaths. ECG-strain was associated with a lower 10-year event-free survival rate for aortic valve replacement or cardiovascular death (log rank test <0.0001; **Figure 6.5**). Indeed, this association persisted even after adjustment for traditional markers of an adverse outcome including the systolic ejection fraction, severity of aortic stenosis, left ventricular mass index and aortic valve calcium score (HR 2.67, 95% confidence interval 1.35 to 5.27; P<0.01; **Figure 6.6**).

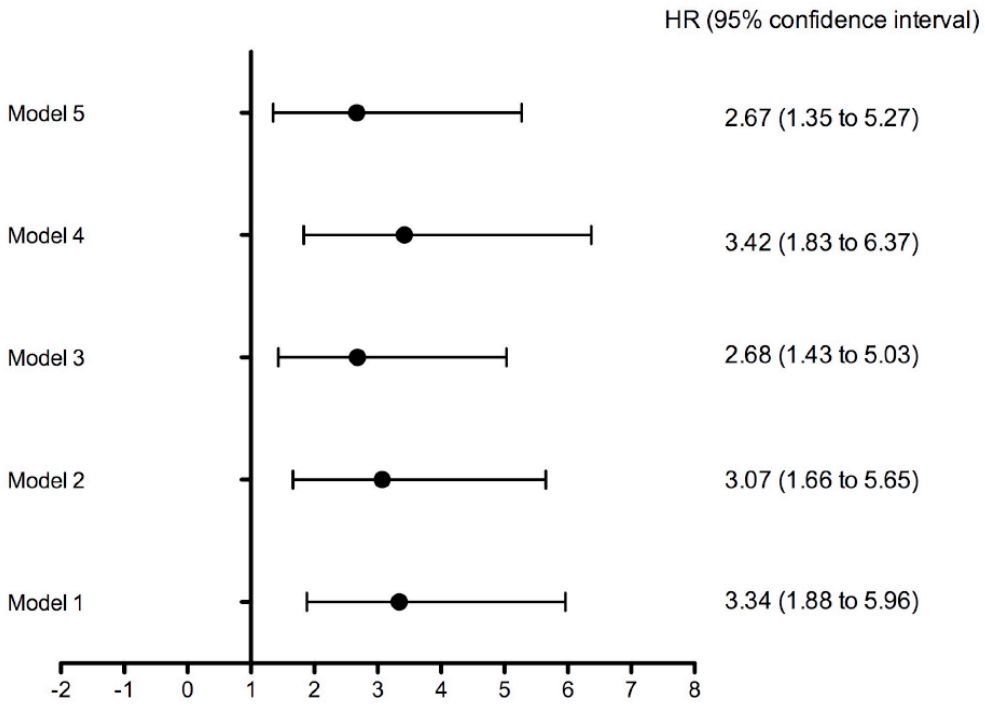
FIGURE 6.5. KAPLAN-MEIER ESTIMATE OF TIME TO EVENT BY STATUS OF ELECTROCARDIOGRAPHIC STRAIN PATTERN IN THE OUTCOME COHORT



No. at risk					
No LVH strain	120	99	76	49	35
LVH strain	20	11	5	3	

Patients with strain pattern on the electrocardiogram had significantly lower event-free survival compared with patients without electrocardiographic strain.

FIGURE 6.6. COX MODELS IN PREDICTING TIME TO ADVERSE EVENTS



Values presented are hazard ratios for the presence of LVH strain in predicting time to aortic valve replacement or cardiovascular death

Model 1: Unadjusted
Model 2: Adjusted for sex and age
Model 3: Model 2 and aortic valve calcium score
Model 4: Model 2, systolic ejection fraction and coronary artery calcium score
Model 5: Adjusted for aortic valve calcium score, mean pressure gradient, systolic ejection fraction and left ventricular mass index

6.5 DISCUSSION

This is the first cardiovascular magnetic resonance study to investigate the mechanisms underlying the ECG-strain pattern in patients with aortic stenosis, demonstrating that it is a highly specific marker of mid-wall myocardial fibrosis. Moreover ECG-strain was associated with increased myocardial injury, impaired left ventricular performance and was an independent predictor of cardiovascular death or aortic valve replacement. Our data therefore indicate that ECG-strain is a powerful biomarker of left ventricular decompensation in aortic stenosis with the ability to identify an at-risk population who may benefit from earlier valve replacement.

Currently, aortic valve replacement is recommended in patients with severe aortic stenosis who have symptoms or evidence of left ventricular decompensation with an ejection fraction <50% (5). However, symptoms are often subjective, particularly in the elderly, whilst a reduced ejection fraction is frequently a late manifestation and not necessarily reversible. There is therefore interest in exploring alternative, earlier and more objective markers of ventricular decompensation in aortic stenosis (95).

Previous echocardiographic studies have demonstrated that ECG-strain is associated with an advanced hypertrophic response (174) and it has been hypothesized that the characteristic repolarization abnormalities relate to coronary perfusion abnormalities, even in the absence of coronary artery disease (75,175-177). Our study adds to these data, demonstrating a close association between ECG-strain and myocardial injury and fibrosis. Indeed, across two independent cohorts, mid-wall myocardial fibrosis was present in 31 of the 32 patients with strain on their ECGs, while the remaining subject had an extensive infarct to explain the ECG changes. Moreover, patients with strain had evidence of higher plasma cTnI concentrations and worse myocardial function. It has been established that myocardial ischemia, cell death and fibrosis are all key features that characterize the transition from hypertrophy to heart failure in aortic stenosis. Our study would therefore support ECG-strain as a useful marker of left ventricular decompensation in

patients with this condition.

In our Outcome Cohort, we have demonstrated that ECG-strain acts as a strong independent predictor of aortic valve replacement or cardiovascular death, over and above established prognostic markers such as systolic ejection fraction, severity of aortic stenosis, left ventricular mass index and aortic valve calcium score. Indeed, patients with ECG-strain had more than a two-fold increase risk in adverse events compared to patients without. This concurs with previous studies that have demonstrated an adverse prognosis associated with ECG-strain (8,178,179). However, our study provides much longer periods of follow up than have been described previously.

There are clear potential advantages of using ECG-strain as a marker of left ventricular decompensation in aortic stenosis. A 12-lead ECG is readily available, cheap and rapidly interpretable. However, whilst ECG-strain is an extremely specific marker for myocardial fibrosis, it is less sensitive. Indeed, in our Mechanism Cohort, more than 30% of patients with replacement myocardial fibrosis did not have strain on the ECG. Importantly these patients had 40% less replacement fibrosis compared to those with strain, suggesting that strain is a relatively late manifestation and that cardiovascular magnetic resonance offers even more sensitive detection of myocardial fibrosis and left ventricular decompensation.

Our data suggest that patients with ECG-strain who are asymptomatic would derive long-term benefit from early aortic valve replacement, due to the prevention of progressive myocardial fibrosis and injury that would otherwise develop whilst waiting for the onset of symptoms. The stage is now set for randomized controlled studies to investigate this strategy, examining the clinical utility of the ECG-strain pattern in guiding early aortic valve replacement alongside other novel and more sensitive markers of left ventricular decompensation including high-sensitivity cTnI concentrations and mid-wall late gadolinium enhancement (58,180).

6.5.1 Study Limitations

In this study, separate cohorts were used to investigate the mechanism and prognosis of patients with ECG-strain because cardiovascular magnetic resonance was not available in the original SALTIRE study. We therefore cannot directly confirm that ECG-strain was similarly related to myocardial fibrosis in the Outcome study. However, ECG-strain in this population did demonstrate the same associations with increased left ventricular mass index, aortic stenosis severity and plasma cTnI concentrations, as observed in the Mechanism Cohort. Moreover in our Validation Cohort, the same clear association between ECG-strain and mid-wall late gadolinium enhancement was also observed. We are therefore confident that ECG-strain acts as a specific marker of mid-wall myocardial fibrosis and left ventricular decompensation in the predominantly Caucasian patients investigated in this study, although further studies will be required for confirmation in different ethnic groups.

6.6 CONCLUSIONS

In patients with aortic stenosis, ECG-strain is a specific marker of mid-wall myocardial fibrosis and an independent predictor of cardiovascular death or aortic valve replacement. Future research should now examine whether the ECG-strain should be used as a marker of left ventricular decompensation to guide early aortic valve replacement in asymptomatic patients.

CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

7.1 SUMMARY OF FINDINGS

Aortic stenosis is a condition that affects both the aortic valve and the myocardium. Advanced multi-parametric cardiovascular magnetic resonance has significantly increased the accuracy and precision of ventricular volumes and mass measurements, and has crucially improved tissue characterization of the myocardium. In this thesis, I have highlighted the importance of looking beyond the valve, demonstrating the effects of stroke volume estimation on the classification of aortic stenosis severity (particularly those with discordant small aortic valve area and low mean pressure gradient aortic stenosis), and the adverse prognosis associated with markers of myocardial fibrosis. Furthermore, I have optimized the novel application of myocardial T1 mapping in aortic stenosis.

7.1.1 Stroke Volume Assessment and Discordant Small-area Low-gradient Aortic Stenosis

Discordant small aortic valve area low mean pressure gradient (small-area low-gradient) aortic stenosis is well described in patients with impaired systolic ejection fraction (7). As a result of reduced cardiac output from the failing heart, the opening of the aortic valve is incomplete. Therefore, the aortic valve area calculated from the continuity equation is smaller than expected and transvalvular gradient measured is low. In recent years, discordant small-area low-gradient aortic stenosis is also increasingly recognized in patients with preserved systolic ejection fraction (**paradoxical low-flow low-gradient aortic stenosis with preserved left ventricular ejection fraction**) and this entity may have important prognostic significance (132-135). Despite normal ejection fractions, stroke volumes are paradoxically reduced in these patients, thought to be the result of pronounced concentric remodeling and small cavity sizes (64,181). Although the inherent limitations of echocardiography and inconsistent thresholds in current guidelines are recognised causes of discordant small-area low-gradient, the significance of these effects on aortic stenosis classification has not been well demonstrated. In my study consisting of 166 patients with aortic stenosis and healthy individuals, I have systematically demonstrated that the magnitude of stroke volume underestimation (and therefore, aortic valve area underestimation) by echocardiography is not trivial. Indeed, the combination of this underestimation and inconsistent thresholds accounted for close to 50% of patients with discordant small-area low-gradient aortic stenosis. After reclassification using stroke volume estimated from cardiovascular magnetic resonance and consistent thresholds, only 5 out of 29 patients with discordant small-area low-gradient aortic stenosis had reduced stroke volumes due to either impaired systolic function or small left ventricles. The remainder consisted of patients with moderate to severe disease, suggesting this represents an entity in transition from moderate to severe disease. The findings also provide a possible explanation for the variable outcomes in different studies of such patients (132-135). Further studies are now needed to investigate the long-term outcomes of patients

reclassified using this approach. Interestingly, other echocardiographic approaches, such as indexed aortic valve area, dimensionless index or stroke volume estimation using the Teichholz formula, did not improve aortic stenosis classification.

7.1.2 Optimization of Myocardial T1 Mapping in Aortic Stenosis

The limitations related to aortic stenosis classification highlighted earlier, alongside significant heterogeneity in the magnitude of hypertrophy in response to similar degrees of aortic valve narrowing, underscore the importance of assessing the myocardium in addition to the aortic valve. Myocyte death and myocardial fibrosis are key processes mediating the transition from compensatory left ventricular hypertrophy to decompensation (36). Therefore, there is considerable interest in markers associated with this transition so as to identify high-risk individuals before heart failure ensues. Indeed, emerging data have demonstrated the adverse prognosis associated with myocardial fibrosis (103-106,182,183). Late gadolinium enhancement is a well-established cardiovascular magnetic resonance technique of assessing focal replacement fibrosis, but it is not well suited for assessing interstitial myocardial fibrosis, which has a more uniform pattern of distribution and predominates in conditions such as aortic stenosis (44). Instead, novel myocardial T1 mapping techniques have been developed to overcome these inherent limitations in late gadolinium enhancement (107). However, the optimal approach has not been established in aortic stenosis, particularly at 3T.

In 40 patients with aortic stenosis and healthy volunteers, I have carefully characterized the temporal and regional profiles of four commonly used T1 measures: pre-contrast T1, post-contrast T1, partition coefficient and extracellular volume fraction. There was no variation in any of the T1 measures across the 16 segments of the left ventricle in both healthy volunteers and patients with aortic stenosis. Both partition coefficient and extracellular volume fraction did not vary with time, while post-contrast T1 was relatively constant only after 15 min. However, only partition coefficient and extracellular volume fraction demonstrated excellent reproducibility (including scan-rescan repeatability) and both measures were significantly higher in asymptomatic patients with aortic stenosis compared with control individuals. This is particularly important because myocardial fibrosis is a slow and progressive process; therefore, a sensitive measure will be critical

to detect any small changes over time. Extracellular volume fraction translates partition coefficient into a percentage of the myocardium affected by diffuse fibrosis and it has a theoretical advantage over partition coefficient because it also corrects for the effects of plasma volume, which can vary from day to day.

7.1.3 High-sensitivity Cardiac Troponin I as a *Sensitive* Marker of Myocardial Fibrosis

Although cardiovascular magnetic resonance has great potential in risk stratifying patients, the clinical utility can be limited by cost, availability and patient suitability. Therefore, my research has also explored suitable biomarkers for clinical use.

Cardiac troponin is a structural protein present in the cardiac myocytes. Increased cardiac troponin concentrations have traditionally been considered to be a specific marker of myocardial necrosis in patients with acute coronary syndromes (115) and recent advances in assay technology have substantially improved sensitivity, allowing quantification of troponin concentrations at very low levels and with a high degree of precision. This has allowed the detection of myocardial injury in a wide range of cardiac conditions, including aortic stenosis. In more than 250 patients with aortic stenosis, I have demonstrated that high-sensitivity cardiac troponin I concentrations were independently associated with measures of left ventricular hypertrophy and myocardial fibrosis on cardiovascular magnetic resonance. Importantly, increased cardiac troponin I concentrations were independently associated with adverse cardiovascular events over more than 10 years of follow-up. Because of the increased assay sensitivity, it was not surprising to find considerable overlap in cardiac troponin I concentrations between patients with aortic stenosis and control cohort. A future strategy would therefore use high-sensitivity cardiac troponin I as a "gate keeper" for cardiovascular magnetic resonance to confirm myocardial fibrosis and ventricular decompensation in patients with aortic stenosis and elevated or increasing troponin I concentrations.

7.1.4 Electrocardiographic Left Ventricular Hypertrophy with Strain as a *Specific* Marker of Myocardial Fibrosis

There are clear advantages of using electrocardiograms in clinical medicine. They are cheap and easily available. Moreover, in a recent study, a particular electrocardiographic pattern of left ventricular hypertrophy with strain was shown to predict worse outcomes in patients with aortic stenosis (8). In a study of more than 300 patients with aortic stenosis, I have confirmed left ventricular hypertrophy with strain pattern on the electrocardiogram is an independent predictor of aortic valve replacement or cardiovascular mortality over 10 years of follow-up; and across 2 cohorts of patients with aortic stenosis, I have demonstrated these characteristic repolarization abnormalities were highly specific for replacement myocardial fibrosis (specificity of 99% and sensitivity of 54%). Furthermore, these patients had more extensive fibrosis, increased myocardial injury and worse cardiac function, suggesting this electrocardiographic pattern is a marker of advanced ventricular decompensation and that cardiovascular magnetic resonance may offer more sensitive detection of myocardial fibrosis and decompensation.

7.2 FUTURE DIRECTIONS

Myocardial fibrosis is associated with adverse prognosis and this risk persists even after aortic valve replacement (51,58,103-106,184). However, most of these studies were small and observational. Furthermore, the association between aortic valve replacement and regression of myocardial fibrosis is conflicting (51,104) and more crucially, the benefits of early aortic valve replacement in asymptomatic high-risk patients with aortic stenosis remain untested.

To date, we have one of the largest prospective cohorts of patients with aortic stenosis and cardiovascular magnetic resonance, designed to specifically address these critical gaps in knowledge. In addition to comprehensive cardiovascular magnetic resonance at baseline, serial imaging will also be performed at 1- and 2-year follow-up regardless of aortic valve replacement. Using both conventional late gadolinium enhancement and novel myocardial T1 mapping techniques, the study will confirm the prognostic significance of myocardial fibrosis in aortic stenosis and whether this risk can be modified by aortic valve replacement. Prospective follow-up and serial imaging will further our understanding in the natural history of the hypertrophic response in patients with and without aortic valve replacement. This knowledge will be essential to set the stage for a randomised controlled trial to test the benefits of early intervention in asymptomatic patients with aortic stenosis and myocardial fibrosis.

The relatively low prevalence of myocardial fibrosis in patients with aortic stenosis (approximately 30% and predominantly in patients with at least moderate severity), coupled with the limitations of cardiovascular magnetic resonance for wide spread clinical use, emphasizes the need for more cost effective markers to identify patients who may benefit from early valve replacement. My future work will develop a clinical risk model based on markers of myocardial fibrosis (such as high-sensitivity cardiac troponin I and electrocardiographic strain pattern), clinical and echocardiographic parameters of aortic stenosis severity. One potential approach could be that

low-risk patients are followed up conservatively with reassessment of risk in subsequent years, and early valve replacement be recommended for high-risk patients. Patients at intermediate risk could be further stratified with cardiovascular magnetic resonance.

The assessment of disease severity and management of patients with aortic stenosis and impaired ejection fraction is extremely challenging and complex. As highlighted earlier, recovery of myocardial function in patients with severe aortic stenosis and impaired systolic ejection fraction may be limited after aortic valve replacement because of irreversible myocardial damage. Whilst evidence of contractile reserve with dobutamine stress echocardiography can predict myocardial function recovery and better outcome after aortic valve replacement, the converse is not always true (64,185). In patients without contractile reserve, myocardial function recovery and prognosis were as good as those with contractile reserve if they survive replacement surgery (186,187). These findings underscore the urgency of more accurate predictors of myocardial function recovery, particularly relevant in the recent years where transcatheter aortic valve implantation has emerged as an alternative to surgical valve replacement in patients deemed at high or prohibitive surgical risk (188,189). In my future research, I will explore the role of cardiovascular magnetic resonance (particularly the presence and extent of myocardial fibrosis) and high-sensitivity cardiac troponin I in predicting myocardial functional recovery after valve replacement in patients with aortic stenosis and impaired systolic ejection fraction.

7.3 CLINICAL PERSPECTIVES

The indications for aortic valve replacement are clear in patients with severe aortic stenosis and symptoms (angina, syncope or dyspnoea) or impaired systolic function. Without timely aortic valve replacement, the risk of death in these patients is about 2% per month (4,190). Conversely, the management of asymptomatic patients is more controversial. Many advocate conservative management because aortic valve replacement will not improve the overall quality of life since patients are asymptomatic. However, some recent evidence suggest improved outcomes with earlier surgery (10,11). Therefore, the crucial question remains: who will benefit from early valve replacement?

In recent years, there has been a greater appreciation that aortic stenosis is a condition that affects not only the valve but also the myocardium. Indeed the transition from left ventricular hypertrophy to heart failure appears to be a key factor in determining the development of symptoms and adverse events. Current assessments of this transition are limited and interest has surrounded the development of novel biomarkers of left ventricular decompensation.

We have learnt that asymptomatic patients with aortic stenosis are a heterogeneous group, with some at higher risk of adverse cardiovascular events than others. Indeed, the duration of the asymptomatic phase varies widely between individuals and despite the absence of symptoms, there is now substantial evidence to suggest ongoing myocardial damage during this subclinical phase. Although the mechanism of sudden cardiac death in asymptomatic patients with aortic stenosis is not well understood, myocardial fibrosis and increased myocardial injury may be the substrates of lethal arrhythmias, above and beyond the severity of valvular obstruction. We now have in our armamentarium more sensitive measures of systolic dysfunction than the conventional ejection fraction. These include state-of-the art imaging techniques to detect early myocardial fibrosis and high-sensitivity assays to measure myocardial injury related to the increased

afterload and ventricular mass. As the operative risks of aortic valve replacement improve and transcatheter aortic valve implantation techniques advance, there will be considerable interest in these novel markers to identify high-risk asymptomatic patients who may benefit from early aortic valvular replacement to prevent further myocardial damage and sudden cardiac death.

Ultimately, randomized controlled trials will be required to evaluate fully these novel biomarkers of left ventricular decompensation, and whether this translates into improved outcomes in patients with aortic stenosis. Instead of relying on a single marker, an integrative approach consisting of clinical characteristics, multi-modality imaging and blood biomarkers will best guide the need for aortic valve replacement.

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APPENDIX

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Date 28 June 2010
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Dear Prof Newby

Study Title: **The role of fibrosis in the aortic valve and the ventricualr myocardium of patients with aortic stenosis**

REC reference number: **10/S1102/24**
Protocol number:

Thank you for your letter of 24 May 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

NHS
Lothian

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Investigator CV		
Protocol	2	24 May 2010
Gp Letter - Aortic Sclerosis Group	2	24 May 2010
Aortic Stenosis - Pre Surgery	2	24 May 2010
REC application		30 March 2010
Covering Letter		10 February 2010
Letter of invitation to participant	2 (Control)	24 May 2010
GP/Consultant Information Sheets	2 (Control Group)	24 May 2010
Participant Information Sheet: AVR group	2	24 May 2010
Response to Request for Further Information		24 May 2010
Participant Information Sheet: Control	2	24 May 2010
Participant Information Sheet: Aortic Sclerosis Group	2	24 May 2010
Participant Information Sheet: Aortic Stenosis Group	2	24 May 2010

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Participant Consent Form: Control Group	X	2	24 May 2010
Participant Consent Form: Aortic Sclerosis Group	X	2	24 May 2010
Participant Consent Form: Aortic Stenosis Group	X	2	24 May 2010
Participant Consent Form: AVR Group	X	2	24 May 2010
Questionnaire: The Kansa City CArdiomyopathy Questionnaire	X	Validated	
GP Letter - Aortic Stenosis Group	X	2	24 May 2010
GP Letter - AVR Group	X	2	24 May 2010
Aortic Sclerosis Invitation Letter	X	2	24 May 2010
Marc Dweck CV			
Aortic Stenosis Group Invitation Letter	X	2	24 May 2010

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

NHS
Lothian

10/S1102/24	Please quote this number on all correspondence

Yours sincerely

Professor Peter Hayes
Chair

Email: lyndsay.baird@nhslothian.scot.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

"After ethical review – guidance for researchers"

Copy to: *Dr Marc Dweck*

NHS
Lothian

South East Scotland Research Ethics Committee 02

Attendance at Sub-Committee of the REC meeting

Committee Members:

Name	*Profession*	*Present*	*Notes*
Professor Peter Hayes	Professor of Hepatology	Yes	
Mr Thomas Russell	Consultant Neurosurgeon	Yes	

The Role of Fibrosis in Aortic Stenosis
PROTOCOL

INTRODUCTION

Aortic stenosis is the most common adult heart valve condition seen in the western world. It represents a significant health burden and is the most common indication for aortic valve surgery. Yet the mechanisms causing this important disease are poorly understood, and there are currently no effective medical treatments capable of altering its course. The only treatment option is surgical replacement of the valve when patients become symptomatic. However, patients are usually elderly and not ideally suited to a major operation. Furthermore we have very few markers that accurately predict prognosis or how quickly aortic stenosis will progress and when surgery will be required.

Given the lack of any form of effective medical therapy for aortic stenosis combined with the absence of reliable markers of disease progression, we believe that it is important to better understand the mechanisms that underlie this condition in order to most effectively develop potential biomarkers and therapy.

Fibrosis plays an important role in the development of aortic stenosis and is known to occur at two different sites: in the aortic valve itself and in the heart muscle. Fibrosis causes the valve to become increasingly stiff which results in the progressive narrowing of the valve orifice that is the hallmark of aortic stenosis.

The muscle of the ventricle has to eject blood through this narrowed valve. This puts an increased strain on the heart, which ultimately can also lead to the development of fibrosis within the heart muscle. Fibrosis in the heart muscle is associated with impaired performance and an adverse prognosis in other cardiac conditions.

We propose to investigate the role of fibrosis both within the valve and the heart muscle using magnetic resonance imaging (MRI). Fibrosis in the heart can be examined by administration of a contrast agent called Gadolinium. Areas of fibrosis can then be visualized by late gadolinium enhancement. In addition physicists at the Royal Brompton Hopsital, London have developed a novel MRI sequence, which should allow us to detect fibrosis with even greater sensitivity.

AIM

To evaluate the role of myocardial and valvular fibrosis in Aortic stenosis using cardiac MRI and both:

A) Late gadolinium enhancement
B) A novel T1 mapping sequence

HYPOTHESES
We hypothesise that:

1) The novel MRI sequence will be able to detect fibrosis of the valve and of the heart muscle with equivalent or greater sensitivity than late gadolinium enhancement.

2) That increased fibrosis of the aortic valve will correlate with the severity of aortic stenosis and predicts its rate of progression.

3) That fibrosis in the heart muscle will predict prognosis and clinical outcome of patients with Aortic stenosis

PATIENT POPULATION (total 240)
We will recruit a total of 240 adults (192 with aortic stenosis) who will be asked to provide formal written consent after being provided with the information sheets enclosed.

This will comprise of:

1) 38 age and sex matched control subjects
2) 10 young healthy controls (35 years and younger)
3) 48 with mild aortic stenosis (peak velocity <3m/s)
4) 48 with moderate aortic stenosis (Peak velocity 3-4m/s)
5) 48 severe asymptomatic aortic stenosis
6) 48 patients with severe symptomatic aortic stenosis due to undergo aortic valve replacement (AVR) within 1 year.

Patients in the control group will just have a baseline assessment to assess valve and myocardial fibrosis and will not be followed up. Patients in the AVR group will undergo a different protocol to the rest of the aortic stenosis patients.

PATIENTS NOT UNDERGOING AVR WITHIN THE FIRST YEAR (n-144)
As described below participants will be followed up for 5 years. This will include 3 clinical assessments: as baseline, after 1 year and after 2 years. After 3, 4 and 5 years patients will be contacted by mail or phone to assess their progress. They will not need to attend the hospital on those occasions. An optional repeat MRI scan will be offered during 1st and 2nd year follow up.

PATIENTS UNDERGOING AVR WITHIN THE FIRST YEAR (n=48)
The protocol will differ for these 48 participants in terms of follow up as described below. Briefly they will have an additional CMR scan after 1 year to assess the role of surgery on the ventricle. In addition at the time of surgery we will retain their aortic valve, which is usually removed and discarded. We will also take small biopsy samples from the heart muscle using a small (3 mm diameter) needle called a tru-cut needle. Two or three passes of the needle will be required to acquire tiny 8 to 15 mg samples from the left ventricular wall. This simple technique safely acquires myocardial tissue as described in highly regarded, peer-reviewed literature (Heymans, Circulation 2005 and Elsasser JACC 2002). The sampling of this tiny amount of

tissue will not affect the function of the heart. Tissue from these biopsies will be analysed under a microscope to measure how much scar tissue (fibrosis) they contain. We can then compare these results to the results from late gadolinium enhancement and the novel MRI sequence. This will allow us to validate the new MRI sequence. An optional repeat MRI scan will be offered during the 2nd year follow up.

EXCLUSION CRITERIA

1. Patients with coexistent moderate aortic regurgitation or mitral stenosis.
2. Patients with acute valvular heart disease e.g. acute mitral regurgitation, active endocarditis.
3. Patients with significant co-morbidities:
    a. severe renal impairment (GFR <30)
    b. liver impairment (INR > 2.0)
4. Patients in acute pulmonary oedema or cardiogenic shock.
5. Patients unsuitable for surgery for non-cardiac reasons e.g. patients with advanced malignancy
6. Patients unable to give informed consent.
7. Patients with significant vasculitis or hypertrophic obstructive cardiomyopathy.
8. Pregnant patients or patients wishing to become pregnant during the timeframe of serial scanning.
9. Patients with a contraindication to undergoing CMR.
10. Age under 18 years

PROTOCOL

BASELINE ASSESSMENT (All patients including controls)

Patients will have a baseline clinical assessment. This will be conducted over the period of a morning or an afternoon and will involve the following aspects. Patients will be given a quality of life questionnaire (Kansas City Cardiomyopathy Questionnaire: see attached) to assess their symptoms and general well being. They will then be asked to perform a six minute walk test where they are asked to walk as far as possible, in six minutes, back and forth between two points 30metres apart. Cardiac MRI will then be performed (using the protocol outlined in detail below) followed by an echocardiogram during which severity of aortic stenosis will be assessed (peak velocity through the valve, peak gradient, aortic valve area). A plastic tube will need to be inserted in to the subject's vein in order to allow administration of the contrast agent during the MRI scan. At this time 30mls of blood will be taken (FBC, U+E, LFT, CRP, BNP, Galectin 3 and other markers of fibrosis: including PIIINP, TIMP-1, TGF-β, PIP, Angiotensin 2, Hyaluronic acid). Finally subjects will be fitted with a Holter monitor, which will monitor their cardiac rhythm for the next 72 hours.

12 MONTH FOLLOW UP (All patients excluding controls)

All patients included in the study will be followed up at one year. It will comprise the same format as the baseline assessment. An MRI will be performed in patients in the AVR group. In all other patients (excluding controls), an optional MRI scan will be offered to patients if they are keen.

2 year follow up (All patients excluding controls)
All patients included in the study will be followed up at two years. It will comprise the same format as the baseline assessment. Cardiac MRI will be offered to all patients during 2nd year of follow-up.

ANNUAL FOLLOW UP FOR 5 YEARS
At 3, 4 and 5 years following the baseline scans participants will be contacted by phone and mail to assess progress (hospital admission, major cardiovascular events). Mortality data will be updated from the office of national statistics, in addition we will check the hospital computer patient records system "TRAK" to ensure that the relatives of patients who have dies are not contacted unnecessarily.

CARDIAC MRI PROTOCOL
Patients will be asked to fill in a safety questionnaire to assess for any contraindication to cardiac MRI scanning. An iv cannula will be inserted and blood samples taken. Patients will be changed in to a gown and ECG stickers placed on their chest. They will then be taken in to the MRI scanner. Patients will be attached to an infusion pump to allow for the injection of contrast and then put inside the scanner.

Basic cardiac MR protocols (axial view – 2 chamber view – 4 chamber view) using black blood imaging will be performed to localize the long and short axis views of the left ventricle (LV). A long axis cine view of LV and short axis cine stack of multiple slices (LV base to apex) will be acquired to allow calculation of ventricular volumes and mass and ejection fractions. Cine views of the aortic valve (2 long axis views and then short axis stack through the valve with no gaps) will also be taken to allow for planimetry of aortic valve area, and flow velocity images will be taken to allow for measurement of peak velocity through the valve.

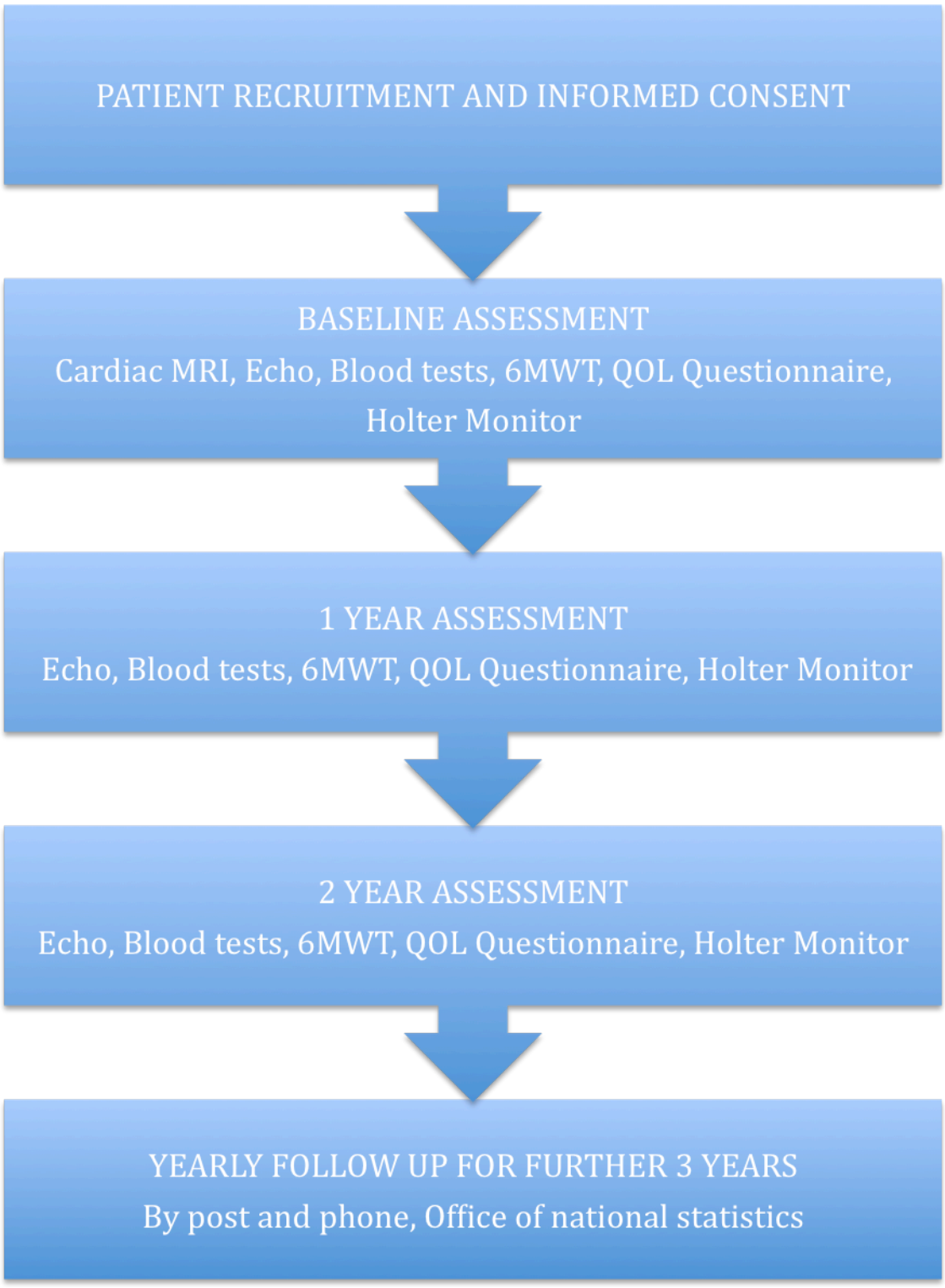
The patient will then receive contrast. 0.1mmol/kg Gadolinium (Gadovist) will be injected using a Medrad Spectris MR–compatible pump injector at 3mls/second, followed by a 20ml flush of saline at the same injection rate. We will then perform T1 mapping of the left ventricle using the novel sequence developed by our collaborators at the Brompton, 10 minutes after Gadolinium injection we will acquire inversion recovery prepared spoiled gradient echo images in standard long and short axis views to detect areas of late gadolinium enhancement.

Subjects will be in the scanner for approximately 45 minutes. Patients who tolerate the scan well will be asked if they mind returning 48 hours later after for a further Cardiac MRI scan after administration of another contrast agent called Feraheme. Feraheme is well tolerated and licensed for clinical human use in the treatment of iron deficiency in chronic kidney disease. However it also allows for the detection of inflammation on MRI scanning. It is given as an infusion, which will be medically supervised. We hope to use it to detect areas of inflammation within the valve alongside the fibrosis.

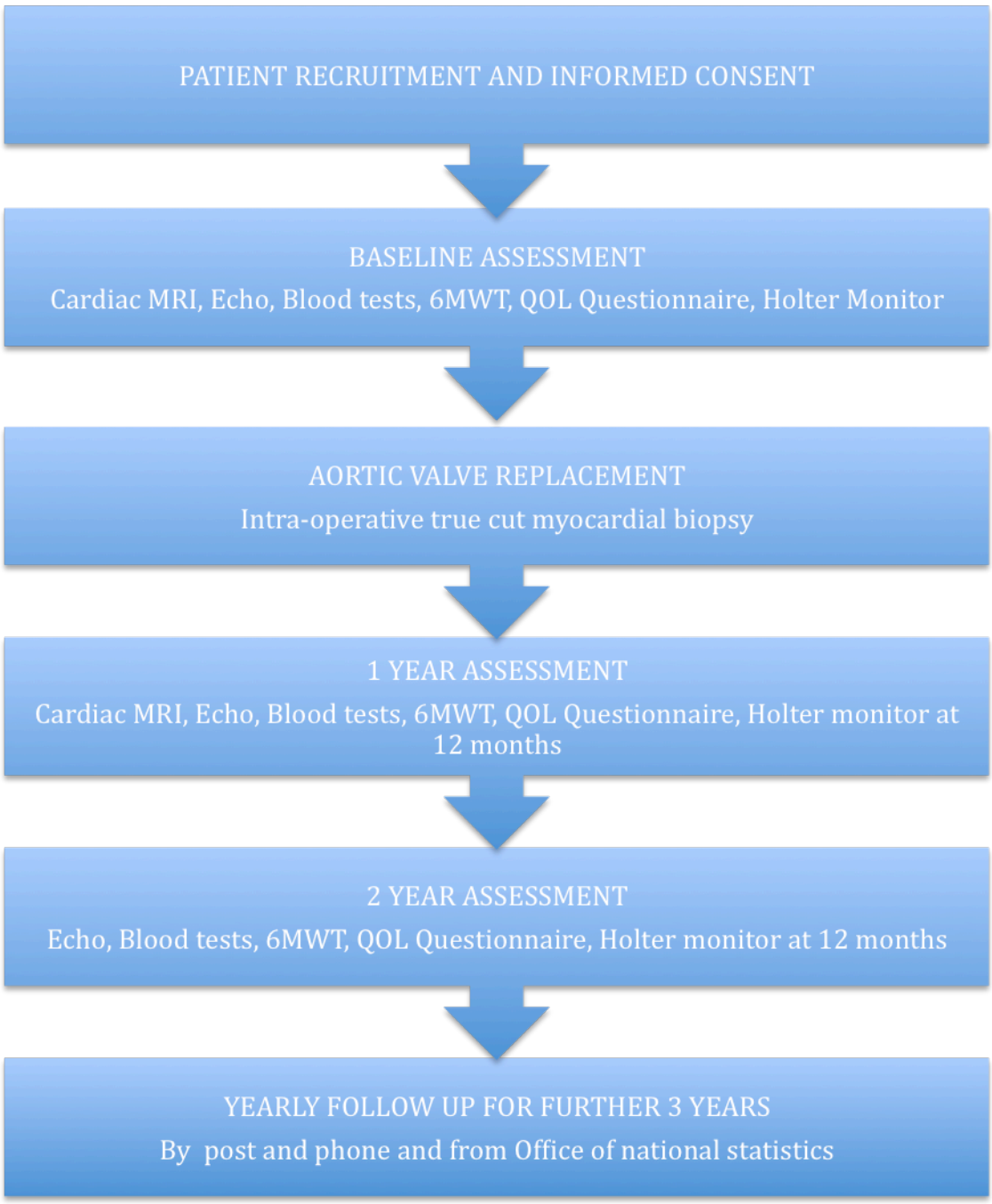
INCIDENTAL FINDINGS

All CMR scans will be reviewed by a consultant radiologist. Any indicental findings made during these scans will be reported to the patient's GP and / or consultant cardiologist as appropriate.

AS Group not undergoing AVR (n=144)



Patients in the AVR group (n=48)



Version 5 18/03/2014

Volunteer Information Sheet
(Aortic stenosis: not undergoing surgery)

ROLE OF FIBROSIS IN THE PROGRESSION OF AORTIC STENOSIS

You are being invited to take part in a research study. Before you decide whether or not to participate, it is important that you understand why the research is being done and what it will involve. Please read the following information and discuss it with others if you wish. If there is anything that you are still unclear about or any questions that you would like to ask you can contact us for further information. You can also contact Dr Bloomfield who is not directly involved with this study but can give you independent advice. He can be contacted through the Royal Infirmary switchboard (0131 242 1000) by asking for his secretary.

Thank you for taking the time to read this information sheet.

Why are we doing this study?

Aortic stenosis is a condition caused by narrowing of one the major valves within the heart. The reasons why this narrowing develops are poorly understood. Using magnetic resonance imaging (MRI) we want to study the role of scarring or "fibrosis" in aortic stenosis, which occurs both in the valve and in the heart muscle. Scarring of the valve contributes to the valve narrowing.

If we understand how the scarring occurs we may be able to develop new treatments to slow or prevent this disease.

Why have I been chosen to take part?

We are looking for patients with aortic stenosis to study using these scans. Using MRI we will assess how much scarring is occurring in your valve and heart muscle. After one year and two years we will ask you to come back for a clinical assessment, after that we will keep in contact yearly by post and phone for 3 years to keep an eye on how you are getting on. By doing the same thing in many patients we will develop a good understanding of how important scarring is in the progression of your disease.

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and you will be asked to sign a consent form. Even once you have signed the form you can still withdraw at any time and you do not have to give a reason.

You will not benefit directly from this study. However, the information we get could help us to improve the way we treat patients with aortic stenosis in the future.

What will happen to me if I decide to take part?

We will need to perform two different assessments of you and your valve. The first will happen when you first agree to participate, the second a year later.

Assessment when you register in the trial

You will be invited to the clinic and the following will happen. We will talk about whether you have any symptoms; ask you to fill in a quality of life questionnaire; perform a full examination of your heart; and organise an echo (ultrasound) scan of your heart. The echo test is not painful and involves no harmful affects to you. You will already have had one of these scans in the clinic. We will then ask you to walk between two points 30 metres apart. The idea is that you walk as far as possible back and forth for 6 minutes.

You will then have an MRI scan of your heart. We will put a plastic tube (cannula) in to your arm so that we can give you the contrast agent, Gadolinium. At the same time we will take some blood from the cannula. If you like you can go in to a model of the MRI machine to check you will be happy lying in it: occasionally people can feel a bit claustrophobic. We will then perform the MRI scan: you will lie on a bed that moves into the scanner. You will lie in the scanner for about 45minutes. You will need to follow some very simple breathing instructions from time to time. It is not painful.

Finally when the MRI scan is finished we will ask you to wear an ECG recorder for 72 hours to check for any underlying problems with the rhythm of the heart. If you tolerate the MRI scan well then we may ask you to come back after 2 days for another MRI scan. This time we will give you another contrast agent via a cannula, which looks at inflammation rather than fibrosis. This contrast agent is safe and used in everyday clinical practice as a treatment for iron deficiency. If you would rather have just the one MRI scan then that is fine.

Assessment after 1 year

This will involve exactly the same assessment as during your first visit. You may be asked to undergo a repeat cardiac MRI. However, this is an optional investigation.

Assessment after 2 yeara

This will involve exactly the same assessment as during your first visit. You may be asked to undergo a repeat cardiac MRI. However, this is an optional investigation.

Yearly assessment at 3, 4 and 5 years

We will contact you by post or mail each year to see how you are getting on. You will no need to come back up to the hospital other than for your routine clinical appointments.

Heart Failure Questionnaire

We will ask you to complete a simple questionnaire that usually applies to patients who have heart failure. Some patients with aortic stenosis may develop symptoms of heart failure in the end stages. We understand that you may not have any of these symptoms but would be grateful if you could complete the questionnaire so that we can compare the answers to the other patients in the study.

What happens to the blood samples that are taken?
A proportion will be tested right away for various tests. A portion of the blood samples taken will be frozen, and stored in an anonymised manner so that further tests may be performed on it in the future. Any further tests would require future ethical approval.

Is there anything else that I have to do?
There are no restrictions on your lifestyle through participating in this study.

Will taking part in this study affect my treatment?
No – you will still be followed up by your cardiologist as if you were not participating in the trial.

What are the side effects of taking part?
The MRI and echo scans are very safe. All contrast agents are safe. You cannot have them if you have kidney problems or any metal in your body but we will check that you have no contraindications very carefully before allowing you to take part in the study.

What are the possible disadvantages of taking part?
Involvement in the trial will mean you attending for various appointments and scans. Whenever possible we will ensure that these occur during the same visit.

What are the possible benefits of taking part?
There will not be any direct benefit to you of taking part in the study, however it is hoped that the findings of this study will benefit other patients with aortic stenosis in the future. The information from the MRI scans can occasionally provide extra information that may be useful to your cardiologist or your general practitioner. We will make certain that if any such information arises that your cardiologist/ general practitioner will be informed.

Will my GP be informed?
As long as you agree we would like to inform your General Practitioner of your participation in this study.

What if something goes wrong?
If you are harmed by taking part in this research project, there are no special compensation arrangements, although in the case of negligent harm, subjects will be covered by the University of Edinburgh insurance policy. If you are harmed as a result of the study you may have grounds for legal action but you may have to pay for this. If you are not satisfied with any aspect of the way you were approached or your treatment during this study, please contact the Faculty of Medicine at the University of Edinburgh.

Will my taking part in this study be kept confidential?
All the information collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?
Once we have completed the study and analysed the results, we will write a paper which will be submitted for publication in one of the medical journals. We do not routinely contact

participants to inform them of the outcome of the research but would be happy to do so if requested. If you would like a copy of the results please contact Dr Calvin Chin using the contact details listed below. You will not be personally identified in any report/publication.

Who is organising the research?
The study is being organised through the University of Edinburgh. Your doctors will not be paid for including you in this study.

Who has reviewed the study?
The study has been reviewed by the South East Scotland Research Ethics Committee 2.

Where can I obtain further information about the study?
You can get further information from Dr Calvin Chin or Prof Newby who will arrange to meet you. You could also discuss the study with Dr Bloomfield who is another doctor working in this hospital who is not involved in the study and can therefore act as an independent advisor.

Dr Chin can be reached by contacting his mobile on 07535001086; his email cchin03m@gmail.com: or by letter addressed to the Cardiovascular Research Unit, Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 SU4. Prof Newby can be contacted via the hospital switchboard (0131 536 1000).

To speak to Dr Bloomfield ask for to be put through to his secretary at the Royal Infirmary of Edinburgh.

Thank you once again for reading this information sheet.

Volunteer Information Sheet
(Aortic stenosis: undergoing AVR)

ROLE OF FIBROSIS IN THE PROGRESSION OF AORTIC STENOSIS

You are being invited to take part in a research study. Before you decide whether or not to participate, it is important that you understand why the research is being done and what it will involve. Please read the following information and discuss it with others if you wish. If there is anything that you are still unclear about or any questions that you would like to ask you can contact us for further information. You can also contact Dr Bloomfield who is not directly involved with this study but can give you independent advice. He can be contacted through the Royal Infirmary switchboard (0131 242 1000) by asking for his secretary.

Thank you for taking the time to read this information sheet.

Why are we doing this study?

Aortic stenosis is a condition caused by narrowing of one the major valves within the heart. The reasons why this narrowing develops are poorly understood. Using magnetic resonance imaging (MRI) we want to study the role of scarring or "fibrosis" in aortic stenosis, which occurs both in the valve and in the heart muscle. Scarring of the valve contributes to the valve narrowing.

If we understand how the scarring occurs we may be able to develop new treatments to slow or prevent this disease.

Why have I been chosen to take part?

We are looking for patients with aortic stenosis who are about to have an aortic valve replacement. Using MRI we will assess how much scarring or "fibrosis" is occurring in your valve and heart muscle. At the time of surgery we will then take your original valve (which is usually just discarded) and a small bit of heart muscle, and see how much scarring there really is in these bit of tissue. This will allow us to know how good our scans are at detecting scarring.

After 1 year we will perform a clinical assessment and further MRI scan to see if the amount of scarring has changed. After 2 years we will repeat the clinical assessment only. We will then keep in contact yearly by post and phone over the next 3 years to keep an eye on how you are getting on. By doing the same thing in lots of patients we will develop a good understanding of how good our scans are at detecting fibrosis. We will also know how important scarring is in how well patients do after surgery and whether it is reversible. In total the trial will run for five years.

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and you will be asked to sign a consent form. Even once

you have signed the form you can still withdraw at any time and you do not have to give a reason.

You will not benefit directly from this study. However, the information we get could help us to improve the way we treat patients with aortic stenosis in the future.

What will happen to me if I decide to take part?

We will need to perform two different assessments of you and your valve. The first will happen when you first agree to participate, the second a year later.

Assessment when you register in the trial

You will be invited to the clinic for a clinical assessment. The following will happen. We will talk about whether you have any symptoms; ask you to fill in a quality of life questionnaire; perform a full examination of your heart; and organise an echo (ultrasound) scan of your heart. The echo test is not painful and involves no harmful affects to you. You will already have had one of these scans in the clinic. We will then ask you to walk between two points 30 metres apart. The idea is that you walk as far as possible back and forth between these two points for 6 minutes.

You will then have an MRI scan of your heart. We will put a plastic tube (cannula) in to your arm so that we can give you the contrast agent. At the same time we will take some blood from the cannula. If you like you can go in to a model of the MRI machine to check you will be happy lying in it. We will then perform the MRI scan: you will lie on a bed that moves into the scanner. You will lie in the scanner for about 45minutes. You will need to follow some very simple breathing instructions from time to time. It is not painful.

Finally when the MRI scan is finished we will ask you to wear an ECG recorder for 72 hours to check for any underlying problems with the rhythm of the heart. If you tolerate the MRI scan well then we may ask you to come back after 2 days for another MRI scan. This time we will give you another contrast agent via a cannula, which looks at inflammation rather than fibrosis. This contrast agent is safe and used in everyday clinical practice as a treatment for iron deficiency. If you would rather have just the one MRI scan then that is fine.

Aortic valve replacement

You will have your aortic valve replacement as planned. Your original, narrowed aortic valve will be removed. Usually it is discarded, however, we will collect it so that we can look at it under the microscope and see how much scarring is there. Also during the surgery we will take a tiny amount of your heart muscle. We will do this using a small biopsy needle (3mm in diameter) called a tru-cut needle. Two or three passes of the needle will be required to acquire tiny 8 to 15 mg samples from the left ventricular wall. This simple technique safely acquires myocardial tissue and has been used many times before in previous studies. The sampling of this tiny amount of tissue will not affect the function of the heart. We can then study this also under the microscope to see how much fibrosis is present.

Assessment after 1 year
This will involve exactly the same assessment as during your first visit

Assessment after 2 years
This will involve exactly the same assessment as during your first visit. You may be asked to undergo a repeat cardiac MRI. However, this is an optional investigation.

Yearly assessment at 3, 4 and 5 years
We will contact you by post or mail each year to see how you are getting on. You will no need to come back up to the hospital other than for your routine clinical appointments.

Heart Failure Questionnaire
We will ask you to complete a simple questionnaire that usually applies to patients who have heart failure. Some patients with aortic stenosis may also develop symptoms of heart failure in the end stages. We understand that you may not suffer from any of these symptoms but would be grateful if you could complete the questionnaire so that we can compare the answers to the other patients in the study.

What happens to the blood samples that are taken?
A proportion will be tested right away for various tests. A portion of the blood samples taken will be frozen, and stored in an anonymised manner so that further tests may be performed on them in the future. Any further tests would require future ethical approval.

What will happen to my aortic valve?
We will take the valve to the laboratory and initially slice it in to segments. We will then look at these segments under the microscopy to see how much scarring is present. The rest of your valve will then by frozen, and stored in an anonymised manner so that further tests may be performed in the future. Any further tests would require future ethical approval.

What will happen to the biopsy of my heart tissue?
We will take the heart muscle to the laboratory and initially slice it in to segments. We will then look at these slices under the microscopy to see how much scarring is present. The rest of your valve will then by frozen, and stored in an anonymised manner so that further tests may be performed in the future. Any further tests would require future ethical approval.

Is there anything else that I have to do?
There are no restrictions on your lifestyle through participating in this study.

Will taking part in this study affect my treatment?
No – you will still be followed up by your cardiologist as if you were not participating in the trial.

What are the side effects of taking part?
The MRI and echo scans are very safe. All contrast agents are safe. You cannot have them if you have kidney failure or any metal in your body but we will check that you have no contraindications very carefully before allowing you to take part in the study.
Your aortic valve is usually discarded at the time of surgery so us using it will in no way affect your treatment. The biopsy of your heart muscle is only tiny and will not effect how your heart pumps.

What are the possible disadvantages of taking part?
Involvement in the trial will mean you attending for various appointments and scans. Whenever possible we will ensure that these occur during the same visit.

What are the possible benefits of taking part?
There will not be any direct benefit to you of taking part in the study, however it is hoped that the findings of this study will benefit other patients with aortic stenosis in the future. The information from the MRI scans can occasionally provide extra information that may be useful to your cardiologist or your general practitioner. We will make certain that if any such information arises that your cardiologist/ general practitioner will be informed

Will my GP be informed?
As long as you agree we would like to inform your General Practitioner of your participation in this study.

What if something goes wrong?
If you are harmed by taking part in this research project, there are no special compensation arrangements, although in the case of negligent harm, subjects will be covered by the University of Edinburgh insurance policy. If you are harmed as a result of the study you may have grounds for legal action but you may have to pay for this. If you are not satisfied with any aspect of the way you were approached or your treatment during this study, please contact the Faculty of Medicine at the University of Edinburgh.

Will my taking part in this study be kept confidential?
All the information collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?
Once we have completed the study and analysed the results, we will write a paper which will be submitted for publication in one of the medical journals. We do not routinely contact participants to inform them of the outcome of the research but would be happy to do so if requested. If you would like a copy of the results please contact Dr Calvin Chin using the contact details listed below. You will not be personally identified in any report/publication.

Who is organising the research?
The study is being organised through the University of Edinburgh. Your doctors will not be paid for including you in this study.

Who has reviewed the study?
The study has been reviewed by the South East Scotland Research Ethics Committee 2.

Where can I obtain further information about the study?
You can get further information from Dr Calvin Chin or Prof Newby who will arrange to meet you. You could also discuss the study with Dr Bloomfield who is another doctor working in this hospital who is not involved in the study and can therefore act as an independent advisor.

Dr Chin can be reached by contacting his mobile on 07535 001 086; his email cchin03m@gmail.com or by letter addressed to the Cardiovascular Research Unit, Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 SU4. Prof Newby can be contacted via the hospital switchboard (0131 536 1000).

To speak to Dr Bloomfield ask for to be put through to his secretary at the Royal Infirmary of Edinburgh.

Thank you once again for reading this information sheet.

Volunteer Information Sheet (Control group)

ROLE OF FIBROSIS IN THE PROGRESSION OF AORTIC STENOSIS

You are being invited to take part in a research study. Before you decide whether or not to participate, it is important that you understand why the research is being done and what it will involve. Please read the following information and discuss it with others if you wish. If there is anything that you are still unclear about or any questions that you would like to ask you can contact us for further information. You can also contact Dr Bloomfield who is not directly involved with this study but can give you independent advice. He can be contacted through the Royal Infirmary switchboard (0131 242 1000) by asking for his secretary.

Thank you for taking the time to read this information sheet.

Why are we doing this study?
Aortic stenosis is a condition caused by narrowing of one the major valves within the heart. The reasons why this narrowing develops are poorly understood. Using magnetic resonance imaging (MRI) we want to study the role of scarring or "fibrosis" in aortic stenosis, which occurs both in the valve and in the heart muscle. Scarring of the valve contributes to the valve narrowing.

If we understand how the scarring occurs we may be able to develop new treatments to slow or prevent this disease.

Why have I been chosen to take part?
We are looking for patients with normal valves to act as "controls" in this study. Using MRI we will assess how much scarring is occurring in your valve and heart muscle. We do not anticipate to find any scarring in your heart. We can then compare these results to those of scans taken in patients with aortic stenosis and see if there is a difference.

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and you will be asked to sign a consent form. Even once you have signed the form you can still withdraw at any time and you do not have to give a reason.

You will not benefit directly from this study. However, the information we get could help us to improve the way we treat patients with aortic stenosis in the future.

What will happen to me if I decide to take part?
You will need to attend the hospital for one day. We will firstly see you in the clinic and talk about whether you have any symptoms; ask you to fill in a quality of life questionnaire; perform a full examination of your heart; and organise an echo (ultrasound) scan of your heart. The echo test is not painful and involves no harmful affects to you. You will already

have had one of these scans in the clinic. We will then ask you to walk between two points 30 metres apart. The idea is that you walk as far as possible back and forth for 6 minutes.

You will then have an MRI scan of your heart. We will put a plastic tube (cannula) in to your arm so that we can give you the contrast agent, Gadolinium. At the same time we will take some blood from the cannula. If you like you can go in to a model of the MRI machine to check you will be happy lying in it: occasionally people can feel a bit claustrophobic. We will then perform the MRI scan: you will lie on a bed that moves into the scanner. You will lie in the scanner for about 45minutes. You will need to follow some very simple breathing instructions from time to time. It is not painful. Finally when the MRI scan is finished we will ask you to wear an ECG recorder for 72 hours to check for any underlying problems with the rhythm of the heart.

Heart Failure Questionnaire
We will ask you to complete a simple questionnaire that usually applies to patients who have heart failure. We understand that you do not have heart failure but would be grateful if you could complete the questionnaire so that we can compare the answers to the other patients in the study.

What happens to the blood samples that are taken?
A proportion will be tested right away for various tests. A portion of the blood samples taken will be frozen, and stored in an anonymised manner so that further tests may be performed on it in the future. Any further tests would require future ethical approval.

Is there anything else that I have to do?
There are no restrictions on your lifestyle through participating in this study.

Will taking part in this study affect my treatment?
No – you will still be followed up by your cardiologist as if you were not participating in the trial.

What are the side effects of taking part?
The MRI and echo scans are very safe. You cannot have them if you have kidney problems or any metal in your body but we will check that you have no contraindications very carefully before allowing you to take part in the study.

What are the possible disadvantages of taking part?
Involvement in the trial will mean you attending for various appointments and scans. Whenever possible we will ensure that these occur during the same visit.

What are the possible benefits of taking part?
There will not be any direct benefit to you of taking part in the study, however it is hoped that the findings of this study will benefit other patients with aortic stenosis in the future. The information from the MRI scans can occasionally provide extra information that may be useful to your cardiologist or your general practitioner. We will make certain that if any such information arises that your cardiologist/ general practitioner will be informed

Will my GP be informed?
As long as you agree we would like to inform your General Practitioner of your participation in this study.

What if something goes wrong?
If you are harmed by taking part in this research project, there are no special compensation arrangements, although in the case of negligent harm, subjects will be covered by the University of Edinburgh insurance policy. If you are harmed as a result of the study you may have grounds for legal action but you may have to pay for this. If you are not satisfied with any aspect of the way you were approached or your treatment during this study, please contact the Faculty of Medicine at the University of Edinburgh.

Will my taking part in this study be kept confidential?
All the information collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?
Once we have completed the study and analysed the results, we will write a paper which will be submitted for publication in one of the medical journals. We do not routinely contact participants to inform them of the outcome of the research but would be happy to do so if requested. If you would like a copy of the results please contact Dr Calvin Chin using the contact details listed below. You will not be personally identified in any report/publication.

Who is organising the research?
The study is being organised through the University of Edinburgh. Your doctors will not be paid for including you in this study.

Who has reviewed the study?
The study has been reviewed by the South East Scotland Research Ethics Committee 2.

Where can I obtain further information about the study?
You can get further information from Dr Calvin Chin or Prof Newby who will arrange to meet you. You could also discuss the study with Dr Bloomfield who is another doctor working in this hospital who is not involved in the study and can therefore act as an independent advisor.

Dr Chin can be reached by contacting his mobile on 07535001086; his email cchin03m@gmail.com or by letter addressed to the Cardiovascular Research Unit, Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 SU4. Prof Newby can be contacted via the hospital switchboard (0131 536 1000).
To speak to Dr Bloomfield ask for to be put through to his secretary at the Royal Infirmary of Edinburgh.

Thank you once again for reading this information sheet

Version 5 18/03/2014

Volunteer Consent Form
(Aortic stenosis group)

ROLE OF FIRBOSIS IN THE PROGRESSION OF AORTIC STENOSIS

Participant Name	
Participant DOB	
Participant identification No for this trial	

☐ I confirm that I have read and understood the accompanying information sheet.

☐ I agree to take part in the above research project as described in the information sheet.

☐ I understand that this will not influence the medical treatment that is planned for me in any way.

☐ I understand that I am under no obligation to participate in this research, that I may withdraw from the study at any stage and that doing so will not influence my treatment in any way.

☐ I understand that any information related to my case will be stored in an anonymised manner

☐ I consent to my GP being informed as to my participation in the trial.

☐ I understand that blood samples will be stored for future use. I agree to them being used in future studies subject to ethical approval

Signed…… Date ……..

Name of patient ……

I confirm that the nature of the research, and the voluntary nature of the study have been explained in terms understandable to the patient

Signed …….. Date……..

Name of investigator ……..

Version 5 18/03/2014

Volunteer Consent Form (AVR Group)

ROLE OF FIRBOSIS IN THE PROGRESSION OF AORTIC STENOSIS

Participant Name	
Participant DOB	
Participant identification No for this trial	

☐ I confirm that I have read and understood the accompanying information sheet.

☐ I agree to take part in the above research project as described in the information sheet.

☐ I understand that this will not influence the medical treatment that is planned for me in any way.

☐ I understand that I am under no obligation to participate in this research, that I may withdraw from the study at any stage and that doing so will not influence my treatment in any way.

☐ I understand that any information related to my case will be stored in an anonymised manner

☐ I consent to my GP being informed as to my participation in the trial.

☐ I consent to my aortic valve being retained after my aortic valve replacement, and used for in vitro studies.

☐ I consent to a small biopsy of my left ventricle being taken at the time of my aortic valve replacement

☐ I understand that my aortic valve, heart muscle biopsy and blood samples will be stored for future use. I agree to them being used in future studies subject to ethical approval

Signed…… Date ……..

Name of patient ……

I confirm that the nature of the research, and the voluntary nature of the study have been explained in terms understandable to the patient

Signed …….. Date……..

Name of investigator ……..

Version 5 18/03/2014

Volunteer Consent Form
(Control group)

ROLE OF FIRBOSIS IN THE PROGRESSION OF AORTIC STENOSIS

Participant Name	
Participant DOB	
Participant identification No for this trial	

☐ I confirm that I have read and understood the accompanying information sheet.

☐ I agree to take part in the above research project as described in the information sheet.

☐ I understand that this will not influence the medical treatment that is planned for me in any way.

☐ I understand that I am under no obligation to participate in this research, that I may withdraw from the study at any stage and that doing so will not influence my treatment in any way.

☐ I understand that any information related to my case will be stored in an anonymised manner

☐ I consent to my GP being informed as to my participation in the trial.

☐ I understand that blood samples will be stored for future use. I agree to them being used in future studies subject to ethical approval

Signed…… Date ……..

Name of patient ……

I confirm that the nature of the research, and the voluntary nature of the study have been explained in terms understandable to the patient

Signed …….. Date……..

Name of investigator ……

EXPERT REVIEWS

Markers of left ventricular decompensation in aortic stenosis

Expert Rev. Cardiovasc. Ther. Early online, 1–12 (2014)

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Calcified aortic stenosis is a condition that affects the valve and the myocardium. As the valve narrows, left ventricular hypertrophy occurs initially as an adaptive mechanism to maintain cardiac output. Ultimately, the ventricle decompensates and patients transition towards heart failure and adverse events. Current guidelines recommend aortic valve replacement in patients with severe aortic stenosis and evidence of decompensation based on either symptoms or an impaired ejection fraction <50%. However, symptoms can be subjective and correlate only modestly with the severity of aortic stenosis whilst impaired ejection fraction is an advanced manifestation and often irreversible. In this review, the authors will discuss the pathophysiology of left ventricular hypertrophy and the transition to heart failure. Subsequently, the authors will examine novel biomarkers that may better identify the transition from hypertrophy to heart failure and therefore guide the optimal timing for aortic valve replacement.

KEYWORDS: aortic stenosis • cardiac MRI • echocardiography • left ventricular decompensation • left ventricular hypertrophy • myocardial fibrosis • myocardial T1 mapping • myocyte death • tissue Doppler imaging

Calcific aortic stenosis is the most common valvular heart condition in developed countries, displaying an increasing prevalence with age [1,2]. Aortic stenosis is characterized by progressive narrowing of the aortic valve that is driven by a complex, active and highly regulated process of inflammation, fibrosis and calcification that leads to leaflet thickening and immobility [3,4]. While the pathophysiology is in many respects similar to atherosclerosis, the factors driving symptom development and clinical events are different [2,5]. For example, aortic stenosis is characterized by the hypertrophic response of the left ventricle that occurs in response to the progressive valve narrowing. This is initially adaptive, restoring wall stress and cardiac performance, but ultimately this process decompensates and patients progress toward heart failure, symptoms and adverse clinical outcomes [6].

Current guidelines advocate aortic valve replacement in patients with severe valvular stenosis and evidence of left ventricular (LV) decompensation: the latter defined by either the presence of symptoms or an impaired ejection fraction <50% [7,8]. Unfortunately, aortic stenosis commonly occurs in elderly patients with comorbidities (such as coronary artery disease, hypertension and chronic lung diseases) that may confound symptom presentation and contribute to adverse cardiovascular outcomes. Furthermore, an impaired ejection fraction occurs late in the disease process when myocardial damage may not be reversible. While the risk of sudden cardiac death during the asymptomatic phase is relative low (~1% per year in large prospective series [9,10]), it is not negligible. Indeed, recent studies have suggested improved outcomes with early aortic valve replacement in asymptomatic patients with preserved systolic function [11,12].

There is considerable interest in defining more objective and sensitive biomarkers of LV decompensation, so that those at particularly high risk can be identified and offered early surgery. In this review, we will first discuss the pathophysiology of LV hypertrophy in aortic stenosis and how this ultimately progresses to heart failure. We will then review some novel biomarkers of this transition that might have an increasing role in future clinical practice.

Pathophysiology of LV hypertrophy

In LV pressure overload conditions such as aortic stenosis and hypertension, myocyte size

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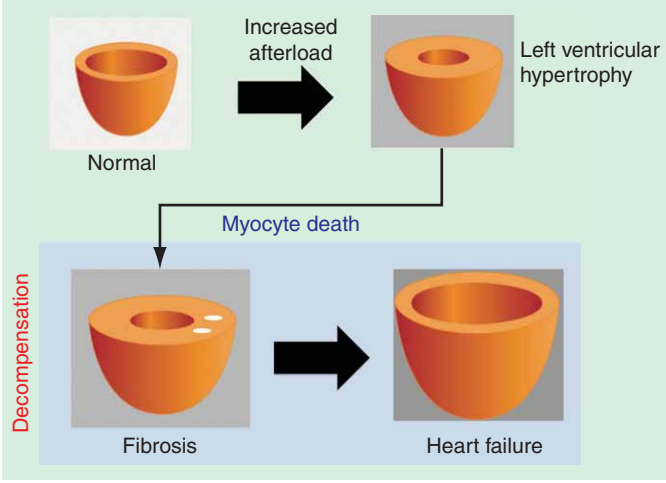

Figure 1. The pathophysiology of left ventricular hyeprtrophy and the transition to heart failure in aortic stenosis. In response to the narrowed aortic valve, left ventricular hypertrophy occurs initially to maintain cardiac output and wall stress. Ultimately, it decompensates and heart failure and other symptoms ensue. The transition from adaptation to heart failure is driven by myocyte death and myocardial fibrosis, mediated by angiotensin II and norepinephrine activation as well as myocardial ischemia from increased afterload and left ventricular mass.

and myocardial wall thickness increase to restore wall stress (σ) according to the LaPlace's Law: $\sigma = [P \times r]/2\,h$, where P is LV pressure, r is LV radius and h is the myocardial wall thickness. The changes in ventricular pressure, radius and wall thickness are therefore initially adaptive, maintaining cardiac output and systolic function (Figure 1) [13].

Interestingly, there is significant heterogeneity in the magnitude of hypertrophy that patients develop in response to similar degrees of aortic valve narrowing. Indeed, multiple studies have shown only a weak correlation between the severity of aortic valve narrowing and LV mass [14–17]. Moreover, approximately 10–20% of patients with severe aortic stenosis have no evidence of LV hypertrophy [17,18]. Sex-related differences partially explain this variation, with women having smaller ventricles and lower myocardial mass compared with men [19–23], potentially as a consequence of differences in sex-related hormones and overall body mass [20,23]. However, other clinical factors are also known to influence the magnitude of the hypertrophic response. These include age, the metabolic syndrome, obesity, angiotensin-converting enzyme insertion/deletion polymorphisms and importantly concomitant hypertension, which imposes an additional load on the left ventricle [16,24–28]. In order to account for both the arterial and valvular load on the left ventricle, a measure of the global LV hemodynamic load (valvulo-arterial impedance, Z_{VA}) has been proposed, with Z_{VA} values >3.5–4.5 mmHg/ml/m^2 providing incremental prognostic value in patients with moderate-to-severe aortic stenosis [29,30]. A more complex ventricular–valvular–arterial coupling model has also been developed to more accurately reflect the dynamic interaction between the left ventricle, the narrowed aortic valve and the arterial system [31]. However, the clinical and prognostic value of this theoretical model remains to be established.

Different patterns of ventricular remodeling and hypertrophy are also well described among patients with aortic stenosis. These are traditionally classified into four groups based upon the myocardial wall thickness, the LV volume and the mass: normal geometry, concentric remodeling, concentric hypertrophy and eccentric hypertrophy [32]. More recently, cardiovascular magnetic resonance (CMR) studies have suggested that asymmetric patterns of remodeling and hypertrophy can also be observed in more than a quarter of patients alongside the traditional patterns described above [15]. However, it remains unclear how patients transition between these different patterns, how they relate to the progression to heart failure and what the clinical consequences of these patterns might be. One interesting related aspect is the so-called paradoxical low-flow low-gradient severe aortic stenosis with preserved ejection fraction. This commonly occurs in patients with concentric remodeling who have increased myocardial wall thickness and small LV volumes. As a consequence, stroke volumes are reduced despite a preserved ejection fraction, resulting in a lower than expected mean pressure gradient in the presence of severe aortic stenosis [33].

The transition from hypertrophy to heart failure

As LV hypertrophy increases, it will ultimately decompensate. This is characterized by progressive impairment in LV performance and the development of symptoms (Figure 1) [14,17,34]. The pathologic change from ventricular adaptation to decompensation is driven primarily by two processes: myocyte death and myocardial fibrosis [35]. Myocyte death is predominantly in the form of proteosomal-mediated autophagy and oncosis (cellular and organelle swelling associated with increased membrane permeability), which occurs alongside more conventional forms of apoptosis [35]. This cell death is believed to be activated by neurohumoral mediators such as angiotensin II and norepinephrine [36–39], and by progressive myocardial ischemia. The latter relates to increased myocardial oxygen demand (due to the increased myocardial mass and afterload) and reduced coronary flow reserve (due to impaired microcirculatory perfusion and inadequate expansion of coronary capillary density despite the absence of coronary artery disease) [40,41].

Myocardial fibrosis is one of the histological hallmarks of end-stage heart failure [42]. The pathogenesis of myocardial fibrosis is complex and the distribution varies, depending on the underlying pathology, although it generally exists in two predominant forms (Figure 2) [43]. Replacement fibrosis commonly occurs late in the disease process, is not believed to be reversible and is characterized by a more localized distribution corresponding to areas of myocyte loss [43,44]. By contrast, interstitial fibrosis is more diffusely distributed, reflecting the more uniform and progressive accumulation of collagen in the interstitium, and is thought to be potentially reversible with targeted therapy. Both types of fibrosis are present in aortic stenosis, occupying up to 30% of the myocardium [13,45,46] and leading to progressive impairment of myocardial relaxation and contraction.

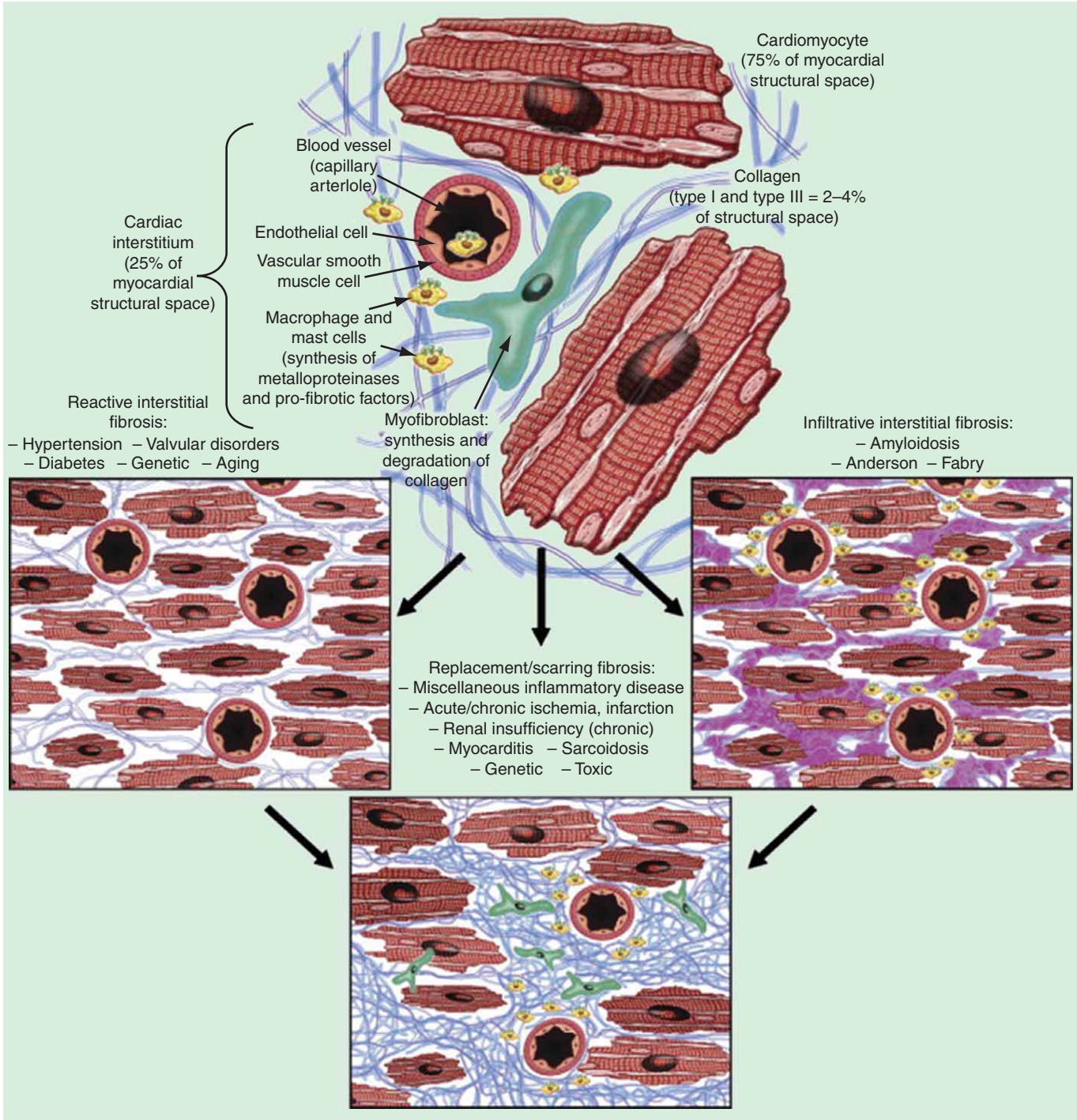

Figure 2. Types of myocardial fibrosis in aortic stenosis. While there are three predominant patterns of myocardial fibrosis, depending on the underlying pathology inflicted on the myocardium, in aortic stenosis two forms predominate: replacement and interstitial myocardial fibrosis. Replacement fibrosis occurs late in the disease process and is characterized by a more localized distribution corresponding to areas of myocyte loss. By contrast, interstitial fibrosis has a more diffuse distribution and appears to be reversible with targeted therapy. Reproduced with permission from [43].

Markers of LV hypertrophy

LV hypertrophy is commonly assessed using echocardiography and the 12-lead electrocardiogram. These are non-invasive, inexpensive and well-tolerated tests, although the latter is relatively insensitive in detecting hypertrophy [47]. By both methods, the presence of LV hypertrophy in patients with aortic stenosis is associated with worse symptoms, impaired systolic function and an adverse prognosis [9,17,48,49].

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In a recent study of 218 asymptomatic patients with aortic stenosis, excessive ventricular mass on echocardiography (defined as measured LV mass exceeding >10% of the predicted value) was associated with a 4.5-fold increase in adverse events, independent of aortic stenosis severity [49]. Although assessments of LV mass by echocardiography are widely used and well studied, they rely heavily upon suitable echocardiography windows and a series of geometrical and mathematical assumptions [32]. This may limit accurate measurements, particularly in subjects with distorted left ventricles or asymmetrical ventricular hypertrophy.

Electrocardiographic evidence of advanced ventricular hypertrophy is similarly associated with an adverse prognosis. Data from the recent Simvastatin and Ezetimibe in Aortic Stenosis substudy demonstrated an independent association between electrocardiographic LV hypertrophy with strain and cardiovascular events in more than 1500 patients and 4 years of follow-up [9]. The exact mechanisms underlying electrocardiographic strain remain unclear, but are related to more severe aortic stenosis, increased LV mass and depressed systolic function, indicating that this inexpensive and widely available test is a marker of an advanced hypertrophic response [50].

CMR imaging is well recognized as the non-invasive reference standard for measuring LV mass, volumes and ejection fraction [51,52]. It is being increasingly used to investigate the hypertrophic response in aortic stenosis and, consistent with the electrocardiographic and echocardiographic data, a recent study observed a trend to the CMR-derived indexed LV mass predicting all-cause mortality on univariate analysis (hazard ratio: 1.01; 95% CI: 1.00–1.02; p = 0.06) [53]. Further studies are required to confirm this finding in larger patient populations.

Markers of LV decompensation

LV performance

Systolic function

The LV ejection fraction is the conventional marker of global systolic dysfunction. Current guidelines recommend aortic valve replacement in patients with severe aortic stenosis and a reduced ejection fraction <50% [7,8]. However, the evidence for using ejection fraction as an indication for aortic valve replacement is weak. Indeed, this recommendation is largely based upon limited retrospective studies that demonstrated an improvement in LV function following aortic valve replacement in patients with severe aortic stenosis and impaired ejection fraction [54,55].

One of the key limitations in using the ejection fraction in aortic stenosis is its tendency to overestimate myocardial systolic function in the presence of advanced concentric hypertrophy. This is because the associated increases in myocardial wall thickness and filling pressures, alongside reductions in ventricular volumes can result in a normal or even supranormal ejection fraction, despite significant impairment in intrinsic myocardial contractility [56–58]. By contrast, echocardiographic assessment of mid-wall fractional shortening and longitudinal function better reflect such contractility. They have been associated with the presence of symptoms and the magnitude of the LV afterload in aortic stenosis, although their prognostic significance remains to be established [59–63]. In addition, novel myocardial deformation imaging (strain and strain rate) using 2D speckle tracking echocardiography has been proposed as an alternative and highly sensitive technique for the assessment of intrinsic myocardial contractility [64,65]. This approach measures the magnitude of myofibril contraction in the left ventricle, which varies in direction according to the different myocardial layers. Indeed, multidirectional strain imaging has demonstrated that myocardial dysfunction is present despite preserved ejection fraction and that it progresses in a step-wise fashion from subendocardial dysfunction in mild aortic stenosis (abnormal longitudinal deformation) to mid-wall dysfunction in moderate aortic stenosis (abnormal circumferential deformation), and eventually transmural dysfunction in severe disease (abnormal radial deformation) [66–68]. This technique also appears to provide prognostic information, with impaired longitudinal myocardial strain and strain rate predicting an adverse outcome in asymptomatic patients with aortic stenosis [69].

Diastolic function

Diastolic dysfunction and impaired LV relaxation occur in aortic stenosis as the left ventricle hypertrophies and becomes fibrosed [13,70–72], frequently preceding reductions in ejection fraction. Current studies examining diastolic dysfunction in aortic stenosis have largely relied on Doppler mitral inflow and myocardial tissue velocities [48,73–75], with limited data using myocardial strain and strain rate imaging. These echocardiographic measures of diastolic dysfunction are associated with worse symptomatic status [48,75], and predict adverse cardiovascular events [30,73,76]. Therefore, they hold potential as early markers of LV decompensation, although their relationship with the more sensitive markers of systolic dysfunction is not well understood and there is some inconsistency with respect to their prognostic value [74].

Measurement of the left atrial size is an alternative method for assessing diastolic function that has been the subject of several small-scale studies [30,76]. It is also closely linked with the development of atrial fibrillation, which in the context of aortic stenosis is associated with advanced hypertrophy, an impaired ejection fraction and an increased risk of heart failure and cerebrovascular events [77].

Role of exercise stress testing

The prompt identification of symptoms is crucial in the effective management of patients with aortic stenosis, given the poor prognosis associated with their development [6]. However, it should be noted that the cardinal symptoms established by Ross and Braunwald (angina, exertional dyspnea, pre-syncope, syncope) were based on young patients with bicuspid or rheumatic disease (average age of 63 years old at time of death) compared with the older patients who present today with

calcific aortic stenosis and comorbidities. The assessment of symptoms in contemporary clinical practice is therefore frequently challenging. Underreporting is common, and patients may unconsciously limit their activities to minimize symptoms. In these situations, exercise stress testing performed under close supervision and with careful monitoring of blood pressure and electrocardiographic changes may be helpful in unmasking otherwise latent symptoms. However, in a meta-analysis of 7 studies and 491 patients with asymptomatic severe aortic stenosis, the sensitivity, specificity, positive and negative predictive values for an adverse cardiac event after an abnormal exercise stress test were only modest at 75, 71, 66 and 79%, respectively [78]. Nevertheless, both the American Heart Association/American College of Cardiology and European Society of Cardiology guidelines recommend that aortic valve replacement be considered in patients who develop exercise-limiting symptoms or an abnormal blood pressure response (defined as an increase in systolic blood pressure of <20 mmHg) on exercise stress testing [7,8,79].

Blood biomarkers of ventricular decompensation

Brain natriuretic peptide & N-terminal proBNP

Interest has surrounded the use of the blood biomarkers brain natriuretic peptide (BNP) and the related N-terminal fragment of proBNP (NT-proBNP) in aortic stenosis. These are endogenous cardiac hormones released in response to increased LV wall stress and are therefore elevated in patients with decompensated LV function. Several studies have demonstrated that their levels increase as patients transition from hypertrophy to heart failure and that they hold promise in assessing patients with equivocal symptoms and severe disease [80–84]. However, the value of measuring BNP and NT-proBNP in patients who are asymptomatic is less certain. In early studies, BNP and NT-proBNP demonstrated a better correlation with clinical outcomes than traditional measures of aortic stenosis severity [83,85,86]. However, two recent studies have questioned their prognostic value, failing to demonstrate an incremental prognostic value when other clinical and echocardiographic measures of aortic stenosis were also considered [82,87]. Of note, patients in the latter two studies were older (79–83 years old vs 68–72 years old in the other studies), hinting at an important limitation of these biomarkers as both BNP and NT-proBNP increase substantially with advancing age independent of the presence of aortic valve disease [88,89]. This lack of specificity in the elderly (the population most commonly affected by aortic stenosis) makes the selection of appropriate thresholds difficult. Moreover, BNP and NT-proBNP lack sensitivity and levels only increase in the later stages of LV decompensation when symptoms and other markers of LV dysfunction are already apparent [90].

High-sensitivity cardiac troponin concentrations

An alternative blood biomarker that appears to be released earlier during the transition from hypertrophy to heart failure is cardiac troponin. Increased cardiac troponin concentrations have traditionally been considered to be a specific marker of myocardial necrosis in patients with acute coronary syndromes [91]. However, recent advances in assay sensitivity allow quantification of plasma cardiac troponin with a high degree of precision at extremely low plasma concentrations [92]. This allows the detection of myocardial injury in a wide range of cardiac conditions aside from acute coronary syndromes, including aortic stenosis. As previously discussed, myocyte death is believed to be one of the key factors driving LV decompensation in aortic stenosis, and this provides a clear rationale for troponin as a cheap and potentially widely available biomarker of this process.

In a recent study, high-sensitivity cardiac troponin T concentrations were detectable in all 57 patients with moderate and severe aortic stenosis. Moreover, these levels correlated positively with LV wall thickness, ventricular mass and the severity of aortic stenosis but interestingly did not appear related to the presence of co-existent coronary artery disease. Furthermore, the highest quartile of high-sensitivity troponin T concentrations was associated with worst 2-year survival rates [93]. We have recently demonstrated similar findings using a high-sensitivity troponin I assay in more than 250 patients and 10 years of follow-up, again showing that high-sensitivity troponin I concentrations were most closely associated with the increased LV mass and myocardial fibrosis on CMR, and that they predicted an adverse outcome [94]. Although these early data are encouraging, further studies involving larger populations are now needed to confirm these findings and to investigate the potential clinical role for high-sensitivity cardiac troponin in aortic stenosis.

Myocardial fibrosis

A second key mediator in the transition from hypertrophy to heart failure is myocardial fibrosis [95]. Recently, non-invasive techniques have been developed using CMR that are capable of directly visualizing and quantifying such fibrosis. These involve the administration of gadolinium contrast, which accumulates in regions of fibrosis and increases signal in these regions. This technique can therefore identify areas of replacement fibrosis in the myocardium, which appear bright in the mid-wall of the left ventricle, in contrast to the surrounding black-appearing normal myocardium (**Figure 3**) [43,96,97]. Importantly, this pattern of fibrosis can be differentiated from that observed with prior myocardial infarction, which is also frequently observed in patients with aortic stenosis.

Several CMR studies have investigated the role of late gadolinium enhancement in this condition. In a recent study consisting of 143 patients with moderate-to-severe aortic stenosis, we demonstrated that the presence of replacement fibrosis was an independent predictor of mortality providing incremental prognostic value over and above that of the ejection fraction. Indeed, patients with myocardial fibrosis had an eightfold increase in all-cause mortality compared with

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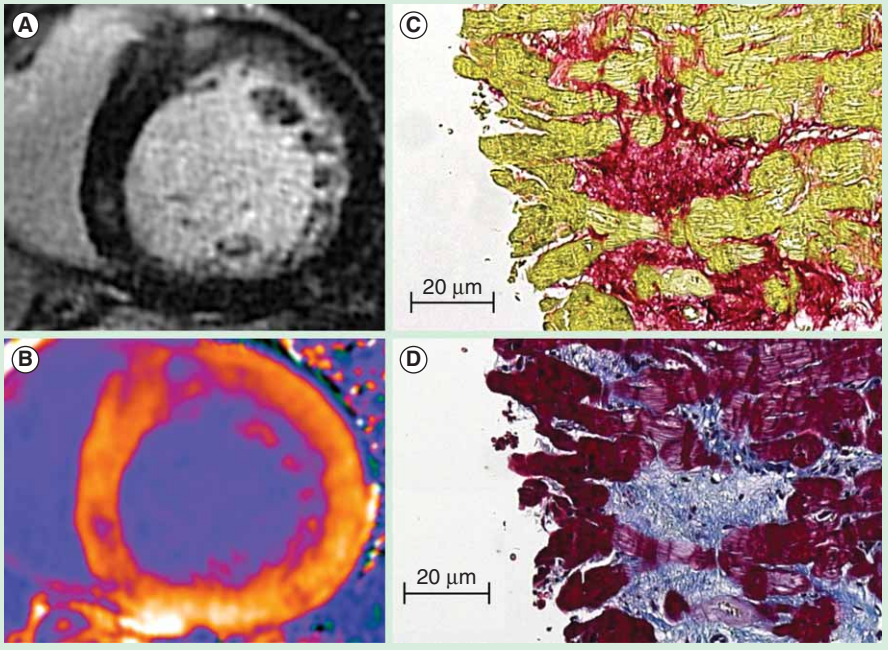

Figure 3. Late gadolinium enhancement imaging and myocardial T1 mapping in a patient with planned aortic valve replacement for severe aortic stenosis. (A) Late gadolinium enhancement imaging demonstrates areas of replacement fibrosis in the basal antero- and infero-septal segments. **(B)** 20 min post-contrast myocardial T1 map of the same basal slice reveals areas of replacement fibrosis, corresponding to the late gadolinium enhanced image. In addition, the extracellular volume fraction calculated in this patient was elevated at 32.7% (the normal extracellular volume in healthy volunteers was 26.0 ± 1.6%). Myocardial biopsy sampled during aortic valve replacement confirms the presence of myocardial fibrosis. **(C)** Collagen fibers stain pink with picrosirius red and **(D)** blue with Masson's trichrome.
Data taken from [105].

those without fibrosis despite similar aortic stenosis severity and coronary artery disease burden [53]. Similar findings have been observed in patients following aortic valve replacement, with the presence of replacement late gadolinium enhancement being associated with adverse ventricular remodeling and worse perioperative and long-term outcomes following aortic valve replacement [98–101]. Interestingly, replacement myocardial fibrosis as detected by late gadolinium enhancement does not appear to be reversible following valve replacement [98], suggesting that once observed in the ventricle, surgery should be considered early before further irreversible fibrosis develops. This hypothesis requires further investigation, but in our opinion late gadolinium enhancement is likely to prove a useful clinical marker of early LV decompensation and indicator for prompt valve replacement.

The predominant form of myocardial fibrosis in aortic stenosis is actually interstitial not replacement fibrosis. This is uniformly distributed through the myocardium and unlike replacement fibrosis, it is thought to be reversible with targeted therapies [102–104]. As a consequence of its diffuse distribution, this form fibrosis is not detected by the late gadolinium enhancement technique, which relies on a difference in signal intensity between normal and fibrotic regions [43]. Instead, novel myocardial T1 mapping approaches have been developed to quantify this form of fibrosis (Figure 3). To date, four major T1 approaches have been assessed and validated against histology with promising results (Table 1). In a recent study, we have compared these different techniques using a standardized and systematic approach, demonstrating that in aortic stenosis the extracellular volume fraction holds the most promise based on its superior reproducibility (±3%) and ability to differentiate patients with this condition from healthy controls [105]. Prospective outcome data with respect to myocardial T1 mapping are lacking in aortic stenosis, although they have been established in other cardiovascular patient populations [106,107].

Two additional novel biomarkers of myocardial fibrosis also deserve mention, although data with respect to aortic stenosis are lacking. Galectin-3 is a member of the lectin family and an important mediator of myocardial fibrosis as demonstrated by a number of experimental studies [108–110]. It has emerged as a potentially useful prognostic marker in patients with heart failure, having recently been associated with all-cause mortality in a community-based study [111–114]. We believe this simple blood test will hold similar promise in aortic stenosis. ^{18}F-Fluciclatide is a PET tracer that has a high affinity for extracellular integrin receptors, thereby acting as a marker of fibrosis activity [115]. Indeed, these integrin receptors are upregulated in states of fibrosis [116,117] and our preliminary data in patients with myocardial infarction indicate that ^{18}F-Fluciclatide activity does indeed map to regions of infarction. A technique capable of measuring fibrosis activity in the myocardium would have potential clinical application in aortic stenosis and further research using ^{18}F-fluciclatide PET is warranted in this condition.

Conclusion

The transition from hypertrophy to heart failure plays a key role in the development of symptoms and adverse events in patients with aortic stenosis. Currently, the identification of this transition and the need for surgery is based on symptoms or a reduced ejection fraction. However, both of these parameters have their limitations and therefore, there is considerable interest in novel biomarkers that can provide earlier and more objective evidence of ventricular decompensation. Further work is required to identify the optimal biomarker or combination

Table 1. Description of commonly used T1 measures in the assessment of interstitial fibrosis.

T1 measure	Characteristics	Advantages	Limitations
Native (non-contrast) T1	Native myocardial T1 values are higher in areas of fibrosis	No contrast required	It measures a composite of both interstitial and myocyte T1, thus it may not be sensitive in less severe myocardial fibrosis
Post-contrast T1	Gadolinium accumulates in areas of fibrosis because of an expanded extracellular volume Post-contrast T1 values are reduced in areas of fibrosis	Contrast improves sensitivity in identifying myocardial fibrosis Can be incorporated in clinical scan easily	Confounded by individual variation in gadolinium kinetics, and timing of imaging Poor scan-rescan reproducibility
Partition coefficient (λ)	Estimates contrast volume of distribution in the interstitial space Expressed as a ratio of T1 signal change in the myocardium and blood pool $\lambda = \Delta R_{myocardium}/\Delta R_{blood\ pool}$, where ΔR = 1/post-contrast T1 – 1/native T1	Excellent scan-rescan reproducibility	Does not account for contrast volume of distribution in plasma
Extracellular volume fraction (ECV)	Similar to partition coefficient, corrects for contrast volume of distribution in the plasma ECV = λ × [1 – hematocrit]	Excellent scan-rescan reproducibility	Hematocrit sampling required in patients Comparison of values across centers may be limited by variabilities in scanners and protocols

of biomarkers that might better guide the timing of aortic valve replacement.

Expert commentary & five-year view

The indications for aortic valve replacement are clear in patients with severe aortic stenosis and symptoms (angina, syncope or dyspnea) or impaired systolic function. Without timely aortic valve replacement, the risk of death in these patients is about 2% per month [6,118]. Conversely, the management of asymptomatic patients is more controversial. Many advocate conservative management because aortic valve replacement will not improve the overall quality of life since patients are asymptomatic. However, some recent evidence suggest improved outcomes with earlier surgery [11,12]. Therefore, the crucial question remains: who will benefit from early valve replacement?

In recent years, there has been a greater appreciation that aortic stenosis is a condition that affects not only the valve, but also the myocardium. Indeed, the transition from LV hypertrophy to heart failure appears to be a key factor in determining the development of symptoms and adverse events. Current assessments of this transition are limited and interest has surrounded the development of novel biomarkers of LV decompensation.

We have also learnt that asymptomatic patients with aortic stenosis are a heterogeneous group, with some at higher risk of adverse cardiovascular events than others. Indeed, the duration of the asymptomatic phase varies widely between individuals and despite the absence of symptoms, there is now substantial evidence to suggest ongoing myocardial damage during this subclinical phase. Although the mechanism of sudden cardiac death in asymptomatic patients with aortic stenosis is not well understood, myocardial fibrosis and increased myocardial injury may be the substrates of lethal arrhythmias, above and beyond the severity of valvular obstruction. We now have in our armamentarium more sensitive measures of systolic dysfunction than the conventional ejection fraction, state-of-the art imaging techniques to detect early myocardial fibrosis and high-sensitivity assays to measure myocardial injury related to the increased afterload and ventricular mass (Figure 4). As the operative risks of aortic valve replacement improve and transcatheter aortic valve implantation techniques advance, there will be considerable interest in these novel markers to identify high-risk asymptomatic patients who may benefit from early aortic valvular replacement to prevent further myocardial damage and sudden cardiac death.

Ultimately, randomized controlled trials will be required to fully evaluate these novel biomarkers of LV decompensation, and whether this translates into improved outcomes in patients with aortic stenosis. Instead of relying on a single marker, we believe an integrative approach consisting of clinical characteristics, novel imaging and blood biomarkers will best guide the need for aortic valve replacement. In particular, high-sensitivity cardiac troponin assays could be used as a screening tool, with patients demonstrating elevated concentrations proceeding to CMR imaging to confirm the presence of mid-wall replacement fibrosis and LV decompensation.

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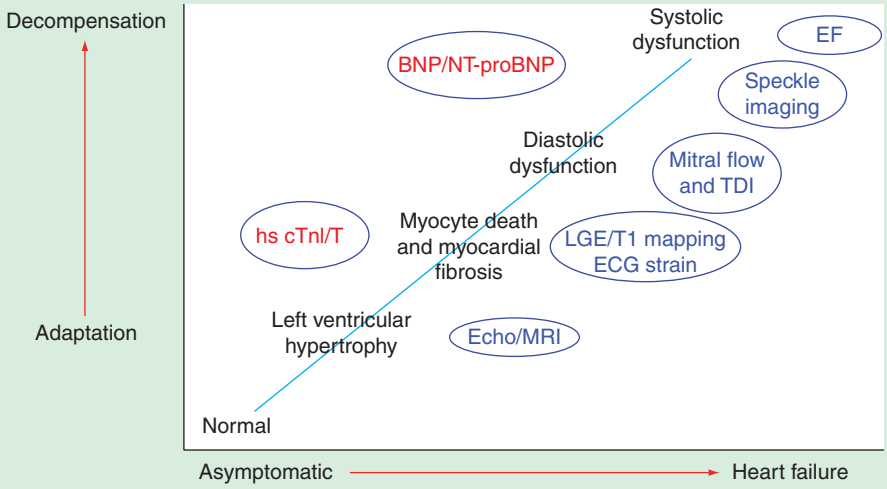

	Marker	Clinical implications
Left ventricular hypertrophy	Increased left ventricular mass on echocardiography and cardiovascular magnetic resonance	Easy to measure. Patients with an advanced response are more likely to transition to heart failure and have an adverse prognosis
Myocyte death and myocardial fibrosis	High sensitivity cardiac troponins	Appears to be a sensitive marker of LV decompensation in aortic stenosis but not specific. Further studies are required to investigate its role in aortic stenosis
	Late gadolinium enhancement and myocardial T1 mapping	Replacement fibrosis assessed using late gadolinium enhancement imaging offers prognostic information in initial studies. It does not appear to reverse after aortic valve replacement. T1 mapping technique detects a different form of fibrosis that appears to occur earlier and is likely to be reversible
	Electrocardiographic left ventricular hypertrophy and strain	Cheap and widely available. It occurs late in the disease process and is associated with an advanced hypertrophic response and markers of LV decompensation. Offers important prognostic information
Diastolic dysfunction	Echocardiographic mitral inflow and tissue Doppler velocities	Easy to measure. However, there are conflicting results with respect to their ability to predict adverse events
Systolic dysfunction	Speckle imaging (strain and strain rate)	Holds potential in detecting early systolic dysfunction before changes in the ejection fraction are apparent
	Brain natriuretic peptide and N-terminal pro-BNP	Increased levels are not manifest until late in the disease process Helpful in patients with equivocal symptoms, but the prognostic value in asymptomatic patients is conflicting
	Ejection fraction	Underestimates intrinsic myocardial systolic dysfunction especially in presence of concentric hypertrophy

Figure 4. A summary of markers of ventricular decompensation associated with aortic stenosis.
BNP: Brain natriuretic peptide; ECV: Extracellular volume; EF: Ejection fraction; LGE: Late gadolinium enhancement; TDI: Tissue Doppler imaging.

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Key issues

- Aortic stenosis is a disease of the valve and the myocardium. In the myocardium, the transition from hypertrophy to heart failure is a key determinant of the development of symptoms and adverse events. This decompensation is mediated by progressive myocyte death and myocardial fibrosis.
- Currently, aortic valve replacement is recommended in patients with severe aortic stenosis and evidence of decompensation, based on either symptoms or impaired ejection fraction <50%.
- Recent studies suggest that early aortic valve replacement improves clinical outcomes in asymptomatic patients with severe disease and preserved systolic function.
- There is emerging interest in novel markers of left ventricular (LV) decompensation to identify patients who may benefit from early aortic valve replacement. These biomarkers include the following:
  - Advanced LV hypertrophy. This can be assessed with different degrees of sensitivity using the electrocardiogram, echocardiography and cardiovascular magnetic resonance imaging and is associated with a worse prognosis.
  - Speckle tracking echocardiography. This is more sensitive than the systolic ejection fraction in detecting intrinsic myocardial dysfunction. Impaired longitudinal strain and strain rate predict an adverse outcome in asymptomatic patients with aortic stenosis.
  - Diastolic dysfunction. This precedes an impaired ejection fraction and is associated with the onset and progression of symptoms. However, there is some inconsistency in the literature with respect to its prognostic role.
  - Brain natriuretic peptide and N-terminal pro-brain natriuretic peptide concentrations. These appear useful in evaluating patients with equivocal symptoms. However, their prognostic value in asymptomatic patients is conflicting.
  - High-sensitivity cardiac troponin concentrations hold potential in detecting the myocyte death that drives the transition from hypertrophy to heart failure. More studies are needed to confirm their prognostic value.
  - Myocardial fibrosis assessment using cardiovascular magnetic resonance. This technique can now be used to identify and quantify the fibrosis driving LV decompensation with initial data suggesting this approach can provide important prognostic data.
- Future research is needed to investigate these novel biomarkers and assess whether they can better identify asymptomatic patients who would benefit from early aortic valve replacement.

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Clinical Research

Echocardiography Underestimates Stroke Volume and Aortic Valve Area: Implications for Patients With Small-Area Low-Gradient Aortic Stenosis

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See editorial by Clavel et al., pages 959-961 of this issue.

ABSTRACT

Background: Discordance between small aortic valve area (AVA; < 1.0 cm^2) and low mean pressure gradient (MPG; < 40 mm Hg) affects a third of patients with moderate or severe aortic stenosis (AS). We hypothesized that this is largely due to inaccurate echocardiographic measurements of the left ventricular outflow tract area ($LVOT_{area}$) and stroke volume alongside inconsistencies in recommended thresholds.

Methods: One hundred thirty-three patients with mild to severe AS and 33 control individuals underwent comprehensive echocardiography and cardiovascular magnetic resonance imaging (MRI). Stroke volume and $LVOT_{area}$ were calculated using echocardiography and MRI, and the effects on AVA estimation were assessed. The relationship between AVA and MPG measurements was then modelled with nonlinear regression and consistent thresholds for these parameters calculated. Finally the effect of these modified AVA measurements and novel thresholds on the number of patients with small-area low-gradient AS was investigated.

RÉSUMÉ

Introduction : La discordance entre une surface valvulaire aortique rétrécie (SVA; < 1,0 cm^2) et un faible gradient de pression moyen (GPM; < 40 mm Hg) touche un tiers des patients souffrant d'une sténose aortique (SA) modérée ou grave. Nous avons posé l'hypothèse que ceci est grandement dû aux mesures échocardiographiques inexactes de la surface de la chambre de chasse du ventricule gauche (surface de la CCVG) et au volume systolique de même qu'à l'incohérence des seuils recommandés.

Méthodes : Cent trente-trois (133) patients souffrant de SA légère à grave et 33 témoins ont subi une échocardiographie complète et une imagerie cardiovasculaire par résonance magnétique (ICRM). Le volume systolique et la surface de la CCVG ont été calculés à l'aide de l'échocardiographie et de l'ICRM, et les effets sur l'estimation de la SVA et les mesures du GPM ont alors été modelés à l'aide de la régression non linéaire et les seuils cohérents de ces paramètres ont été calculés. Finalement, l'effet de ces mesures modifiées de la SVG et des nouveaux

Discordant small aortic valve area (AVA; < 1.0 cm^2), low mean pressure gradient (MPG; < 40 mm Hg) aortic stenosis occurs in approximately 30% of patients with aortic stenosis evaluated using echocardiography.[1,2] This has classically been attributed to patients with low flow states, such as those with reduced left ventricular (LV) ejection fractions.[3] However, in recent years, it has been recognized that small-area low-gradient aortic stenosis can also be observed in the presence of a preserved ejection fraction: so-called "paradoxical low-flow, low-gradient severe aortic stenosis." The outcomes associated with such patients have been variable in different studies,[4-7] presumably reflecting a heterogeneous population and highlighting the uncertainty with regard to the actual severity of aortic stenosis in this subgroup.

Using the continuity equation, the AVA is calculated based on the ratio between the Doppler stroke volume and the post-aortic valve flow. Doppler stroke volume relies crucially on accurate estimation of the LV outflow tract (LVOT) area ($LVOT_{area}$) according to the formula: Doppler stroke volume = $LVOT_{area}$ × LVOT flow. On 2-dimensional echocardiography, the $LVOT_{area}$ is derived from LVOT diameter measurements made on the parasternal long-axis view and the assumption that the LVOT is circular. However, recent experience from transcatheter aortic valve replacement sizing has demonstrated that the LVOT is frequently elliptical and not circular, and as a consequence, measurements made using echocardiography underestimate the true $LVOT_{area}$.[8,9] The implication is therefore that

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Results: Compared with MRI, echocardiography underestimated $LVOT_{area}$ (n = 40; −0.7 cm^2; 95% confidence interval [CI], −2.6 to 1.3), stroke volumes (−6.5 mL/m^2; 95% CI, −28.9 to 16.0) and consequently, AVA (−0.23 cm^2; 95% CI, −1.01 to 0.59). Moreover, an AVA of 1.0 cm^2 corresponded to MPG of 24 mm Hg based on echocardiographic measurements and 37 mm Hg after correction with MRI-derived stroke volumes. Based on conventional measures, 56 patients had discordant small-area low-gradient AS. Using MRI-derived stroke volumes and the revised thresholds, a 48% reduction in discordance was observed (n = 29).

Conclusions: Echocardiography underestimated $LVOT_{area}$, stroke volume, and therefore AVA, compared with MRI. The thresholds based on current guidelines were also inconsistent. In combination, these factors explain > 40% of patients with discordant small-area low-gradient AS.

Résultats : Comparativement à l'ICRM, l'échocardiographie a sous-estimé la surface de la CCVG (n = 40; −0,7 cm^2; intervalle de confiance [IC] à 95 %, −2,6 à 1,3), les volumes systoliques (−6,5 ml/m^2; IC à 95 %, −28,9 à 16,0) et, conséquemment, la SVA (−0,23 cm^2; IC à 95 %, −1,01 à 0,59). De plus, une SVA de 1,0 cm^2 correspondait à un GPM de 24 mm Hg selon les mesures échocardiographiques et de 37 mm Hg après la correction des volumes systoliques issus de l'ICRM. Selon les mesures traditionnelles, 56 patients avaient une SA à surface rétrécie et à faible gradient. À partir des volumes systoliques issus de l'ICRM et des seuils révisés, une réduction de la discordance de 48 % a été observée (n = 29).

Conclusions : L'échocardiographie a sous-estimé la surface de la CCVG, le volume systolique et, par conséquent, la SVA comparativement à l'ICRM. Les seuils des lignes directrices actuelles étaient également incohérents. Combinés, ces facteurs expliquent la raison pour laquelle > 40 % des patients souffrent d'une SA dont la surface rétrécie et le faible gradient sont discordants.

echocardiography might also underestimate the true LV stroke volume and AVA.

In addition, it is widely acknowledged that the severity thresholds for AVA and MPG recommended in current guidelines are inherently inconsistent,[1,10] with theoretical models suggesting an AVA of 1.0 cm^2 corresponds more closely to a MPG of 30-35 mm Hg than the recommended threshold of 40 mm Hg.[10,11]

We hypothesized that the combination of $LVOT_{area}$ underestimation and inconsistent thresholds might influence the classification of aortic stenosis severity, and contribute to the number of patients with discordant small-area low-gradient aortic stenosis. The aims of the study were first to compare stroke volume estimation using echocardiography with the gold standard noninvasive cardiovascular magnetic resonance imaging (MRI) assessment and to establish the optimal thresholds for severe aortic stenosis. Subsequently we then sought to investigate whether correcting for these 2 factors might affect the number of patients with discordant small-area low-gradient aortic stenosis.

Methods

Study participants

Patients with mild to severe aortic stenosis were prospectively recruited from the Edinburgh Heart Centre, and control individuals without aortic stenosis were recruited from the local community. We excluded patients with other significant valvular heart disease (moderate to severe), contraindications to MRI, and cardiomyopathies (inherited or acquired).

The study was conducted in accordance with the Declaration of Helsinki, and approved by the local ethics committee. Written informed consent was obtained from all subjects.

Echocardiography

Transthoracic echocardiography was performed in all patients (iE33, Philips Medical Systems, Best, The Netherlands) by a research ultrasonographer (A.C.W.), and a cardiologist trained in echocardiography (C.W.L.C.). The severity of aortic stenosis was assessed according to the American College of Cardiology/American Heart Association guidelines. Specifically, severe aortic stenosis was defined as an AVA of < 1 cm^2 and MPG > 40 mm Hg.[12] In the parasternal long-axis view, the LVOT diameter was measured at the insertion of the aortic cusps, from the inner edge of the septal endocardium to the inner edge of the anterior mitral leaflet in midsystole (Fig. 1A), because the cross-sectional shape is believed to be more circular at this level.[3] LVOT velocity-time integral was measured in the apical 5-chamber view using pulsed-wave Doppler just proximal to the aortic valve. We were careful to obtain a laminar spectral tracing, avoiding contamination from flow across the aortic valve.

The peak aortic jet velocity and MPG were derived from the aortic valve velocity-time integral, using continuous-wave Doppler. The highest aortic jet velocity and MPG was determined in multiple acoustic windows using standard S51 and D2cwc probes (Philips Medical Systems), and corroborated by the 2 operators. The mean of 3 readings (5 if the patient had atrial fibrillation) was recorded. Doppler stroke volume was estimated ($LVOT_{area}$ × LVOT velocity-time integral) and used to calculate the AVA with the continuity equation (stroke volume/aortic valve velocity-time integral). Normal stroke volume using echocardiography was defined as ≥ 35 mL/m^2.[13] In a further analysis, we had also estimated stroke volume according to the Teichholz method[14] and the effects on aortic stenosis classification.

The severity of aortic valve calcification was assessed in the short-axis view of the aortic valve using a score of 1-4,[15] and corroborated between the 2 operators. Valvuloarterial impedance, a measure of global afterload, was calculated as (systolic blood pressure + MPG)/MRI stroke volume.

MRI

All participants underwent MRI at 3T (Magnetom Verio, Siemens AG, Healthcare Sector, Erlangen, Germany). Cine images were acquired using a balanced steady-state free

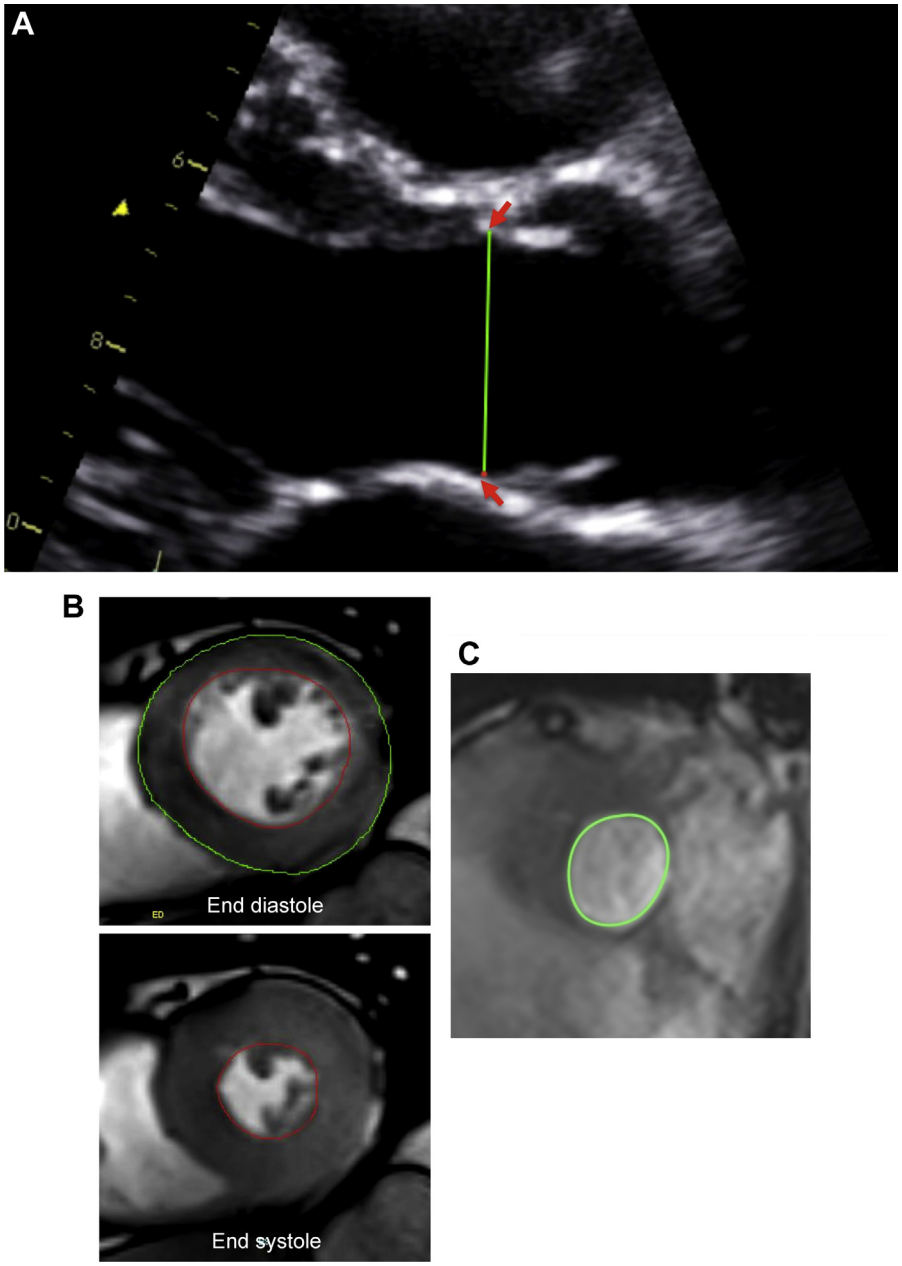

Figure 1. Estimation of left ventricular outflow tract (LVOT) area using echocardiography and magnetic resonance imaging. (**A**) The LVOT diameter was measured at the aortic cusp insertion points (**red arrows**) in the parasternal long axis view. The LVOT area was estimated from the diameter measured. (**B**) The stroke volume was calculated as the difference between end-diastolic and end-systolic volumes. Planimetry of the endocardial borders (**red contours** in end-diastolic and end-systolic frames) was performed including the papillary muscles and minor trabeculations in volume measurements during both phases of the cardiac cycle. Left ventricular mass was calculated by multiplying the total end-diastolic myocardial volume (**green and red contours** in the end-diastolic frame) by the specific gravity of the myocardium (1.05 g/mL). Papillary muscles and minor trabeculations were excluded in mass measurements, with care taken to avoid right ventricular trabeculations. (**C**) Planimetry of the LVOT area in the coaxial short axis view on cardiovascular magnetic resonance imaging at mid-systole.

precision sequence in the short-axis of the left ventricle extending from the atrioventricular ring to the apex (8-mm parallel slices with 2-mm spacing). The endocardial borders were planimetered in end-diastole and end-systole to quantify ventricular volumes and function (Argus, Siemens AG, Healthcare Sector). Papillary muscles and minor trabeculations were included in the volume measurements during both phases of the cardiac cycle as previously described (Fig. 1B).[16,17] Stroke volume was measured as the difference between the end-diastolic and end-systolic LV volumes (in the absence of significant mitral regurgitation), and indexed to body surface area. Normal indexed LV volumes, stroke volumes, and ejection function were defined using sex- and age-specific ranges.[18] LV mass was calculated from the total end-diastolic myocardial volume multiplied by the specific gravity of the myocardium (1.05 g/mL).

Table 1. Baseline characteristics of patients with aortic stenosis and control individuals*

	Control individuals (n = 33)	Aortic stenosis (n = 133)	*P*
Clinical characteristics			
Age, mean years ± SD	54 ± 23	68 ± 12	< 0.01
Male sex, n (%)	18 (55)	89 (67)	0.40
Hypertension, n (%)	9 (27)	85 (64)	< 0.01
Diabetes mellitus, n (%)	0	18 (14)	—
Coronary artery disease, n (%)	3 (9)	44 (33)	0.01
Atrial fibrillation, n (%)	0	3 (2)	—
Echocardiography			
LVOT diameter, cm	2.05 ± 0.17	2.07 ± 0.24	0.66
LVOT cross-sectional area, cm^2	3.30 ± 0.55	3.39 ± 0.85	0.60
LVOT velocity time integral, cm	20.9 ± 3.7	23.5 ± 4.4	0.01
Doppler stroke volume, mL	70 ± 19	79 ± 19	< 0.01
Doppler stroke volume (indexed), mL/m^2	38 ± 8	42 ± 10	< 0.01
Aortic valve area, cm^2	2.36 ± 0.59	0.98 ± 0.40	< 0.01
Aortic valve area (indexed), cm^2/m^2	1.26 ± 0.26	0.52 ± 0.21	< 0.01
Mean pressure gradient, mm Hg	4 ± 1	33 ± 20	< 0.01
Peak aortic velocity, m/s	1.4 ± 0.2	3.8 ± 0.9	< 0.01
Dimensionless index	0.72 ± 0.10	0.28 ± 0.09	< 0.01
Aortic valve calcium score, median (IQR)	1 (1, 1)	3 (3, 4)	< 0.01
Valvuloarterial impedance, mm $Hg/mL/m^2$	3.2 ± 0.7	4.0 ± 1.0	0.34
End-diastolic volume, mL†	93 ± 25	87 ± 26	0.17
End-diastolic volume (indexed), mL/m^2†	50 ± 13	46 ± 13	0.12
End-systolic volume, mL†	41 ± 14	38 ± 14	0.29
End-systolic volume (indexed), mL/m^2†	22 ± 7	20 ± 7	0.16
Stroke volume, mL†	51 ± 16	49 ± 14	0.48
Stroke volume (indexed), mL/m^2†	28 ± 8	26 ± 7	0.16
Ejection fraction, %†	56 ± 9	57 ± 7	0.49
Mild mitral regurgitation, n (%)	2 (6)	19 (14)	0.37
Mild aortic regurgitation, n (%)	2 (6)	57 (43)	< 0.01
Cardiovascular Magnetic Resonance Imaging			
End-diastolic volume, mL	140 ± 32	135 ± 35	0.47
End-diastolic volume (indexed) (EDVi), mL/m^2	75 ± 13	72 ± 16	0.34
End-systolic volume, mL	51 ± 15	46 ± 18	0.14
End-systolic volume (indexed), mL/m^2	27 ± 7	24 ± 9	0.08
Stroke volume, mL	89 ± 19	90 ± 22	0.81
Stroke volume (indexed), mL/m^2	47 ± 8	48 ± 10	0.59
Ejection fraction, %	64 ± 4	67 ± 7	0.02
Left ventricular mass (indexed) (LVMi), g/m^2	67 ± 15	89 ± 22	< 0.01
LVMi/EDVi, g/mL	0.90 ± 0.13	1.25 ± 0.26	< 0.01

EDVi, indexed end diastolic volume; IQR, interquartile range; LVMi, indexed left ventricular mass; LVOT, left ventricular outflow tract.

*Characteristics of patients with aortic stenosis were classified based on aortic valve area estimated using Doppler-derived stroke volume presented in Supplemental Table S1.

†Estimated using the Teichholz formula.

In 40 patients, additional coaxial short-axis cine slices were acquired from the level of the aortic valve. The $LVOT_{area}$ was planimetered at the base of the aortic valve (the slice at which all 3 cusps were first observed to disappear) in midsystole and comparisons were made with the $LVOT_{area}$ estimated from the LVOT diameter on 2-dimensional echocardiography (Fig. 1C).

Curve-fitting and statistical analysis

In patients with normal stroke volumes, the relationship between AVA and MPG was modelled according to the Gorlin equation: $AVA = c/\sqrt{MPG}$ (GraphPad Prism 5, GraphPad Software Inc, San Diego, CA). No rules were set for the initial value for the modelling parameter, *c*. We generated 2 curve-fitting models with AVA derived using Doppler stroke volume and MRI stroke volume.

The distribution of all continuous variables was assessed for normality using the Shapiro-Wilk test, and presented as either mean ± SD or median (interquartile range). Comparison was performed using the Student *t* test or analysis of variance with post hoc Bonferroni adjustment. The Mann-Whitney *U* and Kruskal-Wallis with post hoc Dunn tests were used for nonparametric data. Categorical variables were expressed in percentages and compared using the χ^2 test. The correlation between continuous data was assessed with the Pearson correlation and presented as r^2 values. Comparison between echocardiographic and MRI indices of stroke volume, $LVOT_{area}$, and AVA was assessed using the Bland-Altman analysis. Fixed and proportional biases with 95% limits of agreement were reported. A 2-sided $P < 0.05$ was considered statistically significant.

Results

A total of 133 patients with mild to severe aortic stenosis (AVA, 0.98 ± 0.40 cm^2; MPG, 33 ± 20 mm Hg; peak aortic velocity, 3.8 ± 0.9 m/s) and 33 control individuals were recruited. The median interval between echocardiography and MRI was 9 (interquartile range, 5-29) days. Compared

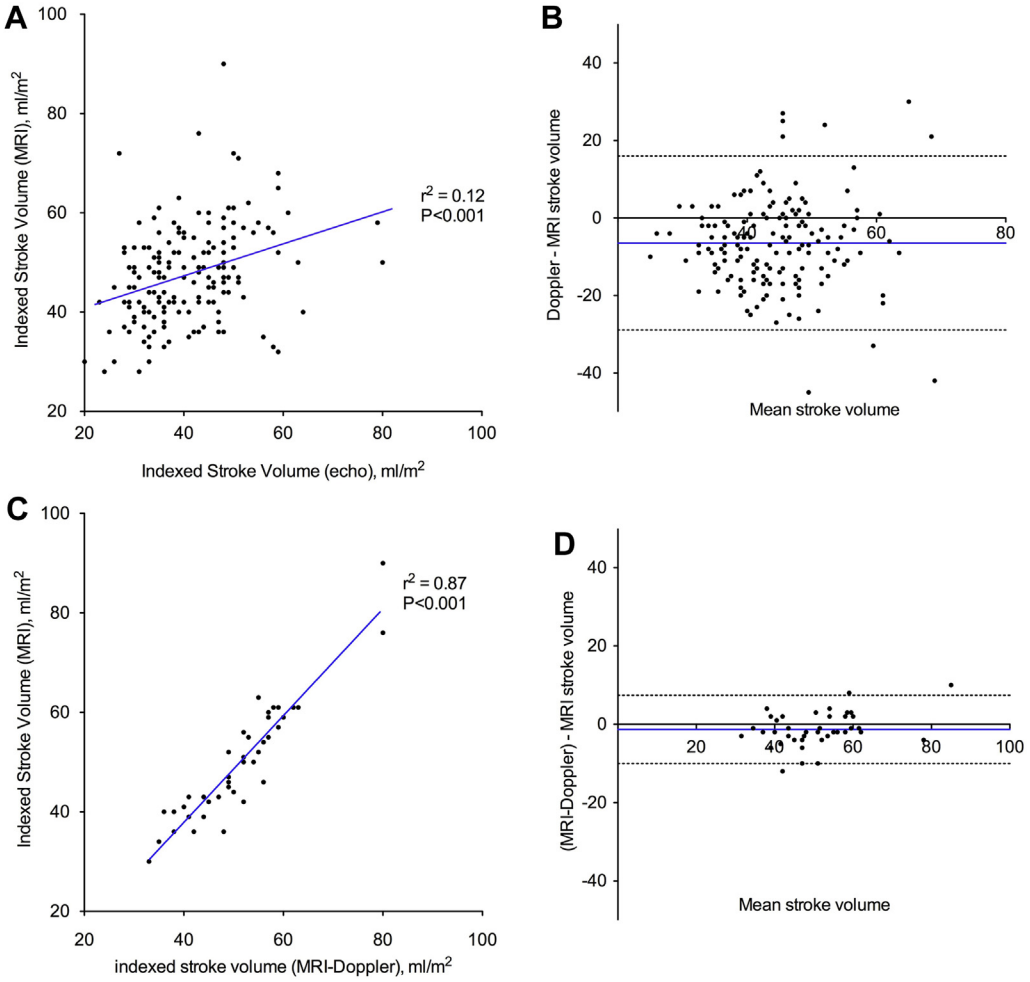

Figure 2. Stroke volume correlation and Bland-Altman analysis. Doppler stroke volume correlated weakly with magnetic resonance imaging (MRI) stroke volume (**A**), with a fixed bias and wide limits of agreement (**B**). In 40 patients, stroke volume was calculated using planimetered left ventricular outflow tract area on MRI and Doppler left ventricular outflow tract flow (MRI-Doppler). This approach demonstrated excellent correlation with MRI stroke volume (**C**), without significant bias (**D**).

with control individuals, patients with aortic stenosis had greater ejection fraction rates (64 ± 4% and 67 ± 7%, respectively; $P = 0.02$) despite similar LV end-diastolic volumes (75 ± 13 mL/m^2 and 72 ± 16 mL/m^2, respectively; $P = 0.34$) and stroke volumes (47 ± 8 mL/m^2 and 48 ± 10 mL/m^2, respectively; $P = 0.59$) (Table 1 and Supplemental Table S1).

Doppler and cardiac MRI stroke volume

Doppler stroke volume correlated only weakly with MRI stroke volume measurements ($r^2 = 0.12$; $P < 0.001$; Fig. 2A) and underestimated the stroke volume by > 6 mL/m^2 compared with MRI (−6.5 mL/m^2; 95% confidence interval [CI], −28.9 to 16.0 mL/m^2; Fig. 2B). Similar results were observed after excluding the 19 patients in the cohort with mild mitral regurgitation ($r^2 = 0.14$; $P < 0.001$; mean difference, −6.1 mL/m^2; 95% CI, −28.2 to 16.0 mL/m^2). This in part appears to be due to underestimation of the $LVOT_{area}$ using echocardiography compared with planimetered $LVOT_{area}$ measurements (−0.7 cm^2; 95% CI, −2.6 to 1.3 cm^2; Fig. 3). Indeed, when we subsequently recalculated stroke volume using the planimetered $LVOT_{area}$, an excellent correlation with MRI stroke volumes was observed ($r^2 = 0.87$; $P < 0.001$; Fig. 2C) without significant fixed or proportional biases (−1.3 mL/m^2; 95% CI, −9.9 to 7.3 mL/m^2; Fig. 2D). Moreover, this effect translated into an underestimation of the AVA calculated using echocardiography-derived stroke volumes compared with MRI-measured stroke volumes (−0.23 cm^2; 95% CI, −1.01 to 0.59 cm^2; Fig. 4). As previously described, the explanation for echocardiographic underestimation of the $LVOT_{area}$ appears related to its elliptic shape. Indeed, the mean ellipticity ratio (ratio of the maximum to minimum LVOT diameter) was 1.2 ± 0.1, with only 28% of these patients having a circular LVOT (defined as ellipticity ratio of 1.0). Of note, we achieved excellent intraobserver ($r^2 = 1.00$; $P < 0.001$; mean difference 0.5 ± 2.7%) and interobserver ($r^2 = 0.98$; $P < 0.001$; mean difference 1.1 ± 5.4%) agreement in the planimetered LVOT measurements using MRI.

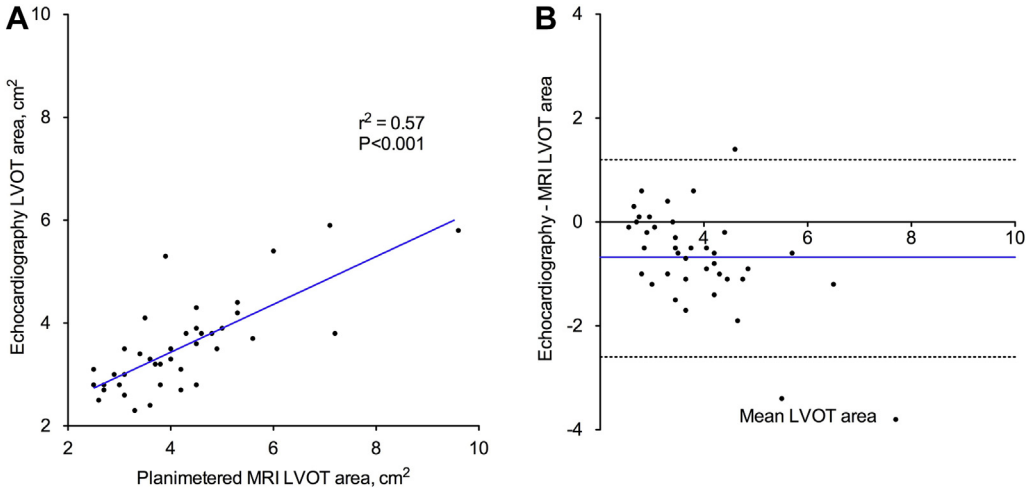

Figure 3. Left ventricular outflow tract (LVOT) area correlation and Bland-Altman analysis. Although LVOT area estimated using echocardiography demonstrated a moderate correlation with planimetered LVOT area on magnetic resonance imaging (**A**), the echocardiographic LVOT area underestimated the planimetered area with wide limits of agreement (**B**).

Consistent AVA and MPG cutoffs

Based on measurements derived from Doppler stroke volume, an MPG of 40 mm Hg corresponded to an AVA of 0.77 cm^2, and an AVA of 1.0 cm^2 corresponded to an MPG of only 24 mm Hg (AVA = 4.85/$\sqrt{}$MPG; r^2 = 0.73; Fig. 5A). When MRI stroke volume measurements were used to calculate the AVA, an MPG of 40 mm Hg corresponded to an AVA of 0.97 cm^2 and an AVA of 1.0 cm^2 corresponded to an MPG of 37 mm Hg (AVA = 6.13/$\sqrt{}$MPG; r^2 = 0.81; Fig. 5B).

Discordant small-area low-gradient aortic stenosis

Using the conventional echocardiographic estimation of MPG and AVA, and the thresholds for severe disease based on current guidelines (AVA, 1.0 cm^2 and MPG, 40 mm Hg),[12,19] 56 patients with aortic stenosis (42%) had discordant small-area low-gradient aortic stenosis (Fig. 6A).

Using a stepwise approach, we first assessed the effect of using AVA measurements derived from MRI stroke volumes on this proportion of patients with discordant small-area low-gradient aortic stenosis. This resulted in 20 patients being reclassified as having nonsevere aortic stenosis (median aortic valve calcium score of 3; valvuloarterial impedance, 3.7 ± 0.7 mm Hg/mL/m^2), leaving 36 with small-area low-gradient aortic stenosis (Fig. 6B). Subsequently, when we used the revised thresholds already described herein (AVA of 1.0 cm^2 and MPG of 37 mm Hg), a further 7 patients were reclassified with severe disease (all had aortic valve calcium score of 4 and valvuloarterial impedance of 4.5 ± 1.2 mm Hg/mL/m^2). This left only 29 patients with discordant small-area low-gradient aortic stenosis, a reduction of 48% compared with the original classification (Fig. 6C). Of these, 3 patients had impaired systolic function and 2 had a low stroke volume due to small LV cavity volumes. The remainder appeared to consist of patients with moderate to severe disease with values for a wide

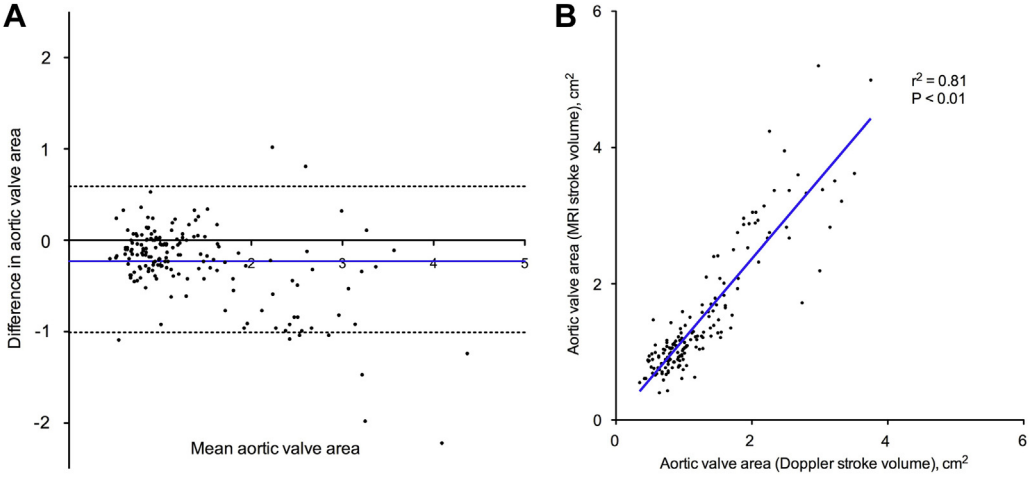

Figure 4. Aortic valve area corrleation and Bland-Altman analysis. Aortic valve area estimated using Doppler stroke volume and magnetic resonance imaging-derived stroke volume demonstrated poor agreement and significant underestimation (**A**), despite excellent correlation (**B**).

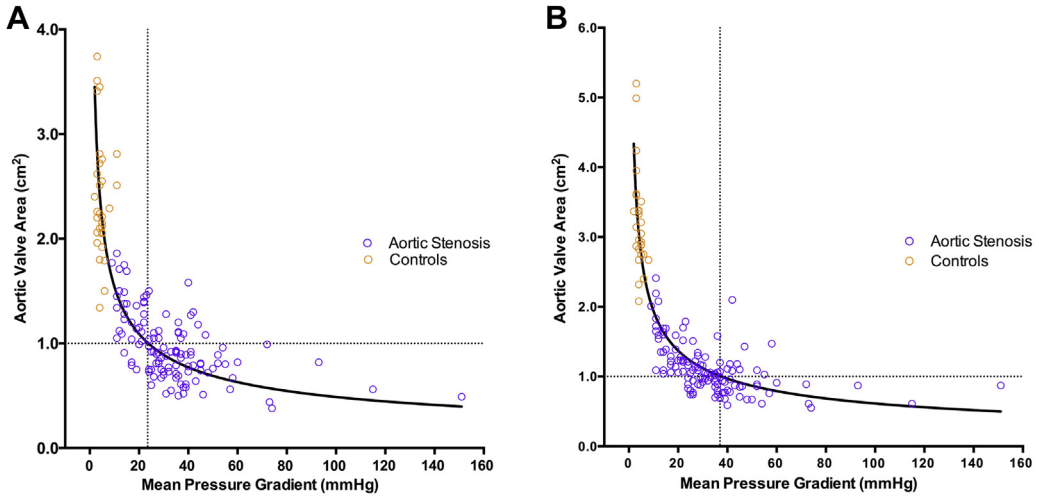

Figure 5. Relationship between aortic valve area and mean pressure gradient. The aortic valve area was calculated from the continuity equation using Doppler stroke volume. An aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of 24 mm Hg (**A**). Correcting these values using the magnetic resonance imaging stroke volume, an aortic valve area of 1.0 cm^2 corresponded to a mean pressure gradient of 37 mm Hg (**B**).

range of parameters that were intermediate between concordant moderate and severe disease (Supplemental Table S2). This included the aortic valve calcium score, which was 3 in 48% and 4 in 52% of patients.

Stroke volume estimation and aortic stenosis classification using the Teichholz formula

In a further analysis, we assessed an alternate echocardiographic method for estimating stroke volumes using the Teichholz formula.[14] Results were similar, with the correlation between echocardiography-estimated and MRI-derived stroke volumes remaining weak ($r^2 = 0.16$; $P < 0.001$), and 51% of patients classified as having discordant small-area low-gradient aortic stenosis.

Discussion

In this study, we have systematically demonstrated that echocardiography underestimates the $LVOT_{area}$, the LV

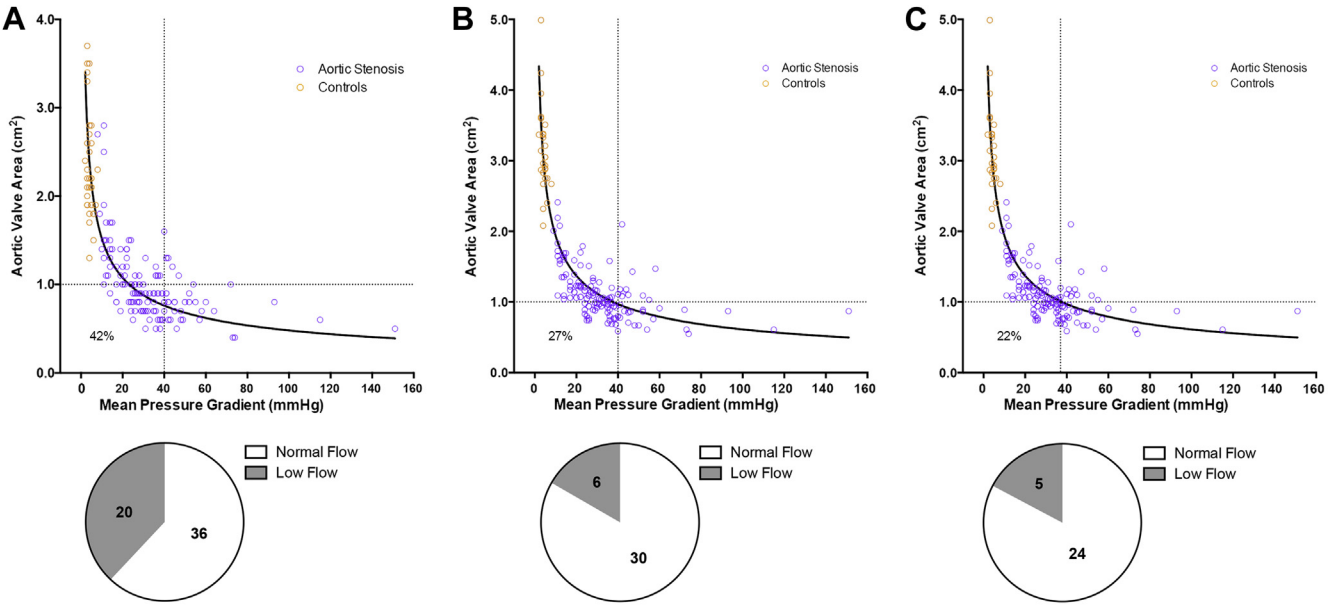

Figure 6. Reclassification of aortic stenosis severity. Using traditional echocardiographic measurements and the recommended severity cutoffs established in current guidelines (**A**), 56 patients had discordant small-area low-gradient aortic stenosis. Twenty patients were reclassified to concordant nonsevere aortic stenosis when cardiovascular magnetic resonance imaging stroke volume was used to estimate aortic valve area (**B**). A further 7 patients were reclassified as having concordant severe disease using the revised thresholds of 1.0 cm^2 and 37 mm Hg (**C**). The corresponding pie charts show the flow states in patients with discordant small-area low-gradient aortic stenosis (stroke volume estimated using cardiovascular magnetic resonance imaging).

stroke volume, and as a consequence, the AVA. Moreover we have demonstrated that there are inconsistencies in the guideline thresholds of severity with an AVA of 1.0 cm^2 corresponding to an MPG of 24 mm Hg based on standard echocardiographic measures and 37 mm Hg when MRI-derived stroke volumes are used. Finally we have shown that if we correct for these 2 factors using the more accurate MRI estimation of the stroke volume to calculate AVA and revised thresholds, more than 40% of the patients with small-area low-gradient aortic stenosis were reclassified as having concordant measurements.

Two-dimensional and Doppler echocardiography assessments are the predominant methods used worldwide to assess the severity of aortic stenosis. However, the echocardiographic estimation of the AVA relies on accurate measurement of stroke volume. Unfortunately as demonstrated in this study, echocardiography frequently underestimated the stroke volume compared with the noninvasive gold-standard measurements made using MRI. As a consequence, echocardiography would also appear to underestimate the AVA. Our data provide explanations for these observations. A subgroup of 40 patients had coaxial short-axis cine images of their LVOT. This allowed accurate and reproducible planimetered measurements of the $LVOT_{area}$ to be compared with the derived measurements made using 2-dimensional echocardiographic diameter measurements. Similar to previous studies,[8,9] we have demonstrated that such echocardiographic measures underestimate the true $LVOT_{area}$ in part because of the fact that the LVOT is frequently elliptical not circular. Indeed, when Doppler stroke volumes were corrected using the more accurate planimetered measurements of the $LVOT_{area}$, a good correlation with MRI-derived stroke volumes was subsequently observed. Further to our analyses, we have also explored using other echocardiographic indices such as indexed AVA and the dimensionless index. Unfortunately, these techniques were also associated with inherent limitations related to the $LVOT_{area}$ measurements (see the *Evaluation of Aortic Stenosis Classification Using Indexed Aortic Valve Area and the Dimensionless Index* section of the Supplementary Material).

Inconsistencies in the MPG and AVA thresholds recommended in the current guidelines are well described.[1,10,20] Consistent with previous reports,[1,10] our echocardiography data confirmed an AVA of 1.0 cm^2 corresponded to a MPG of only 24 mm Hg, significantly lower than the threshold of 40 mm Hg stated in current guidelines. Interestingly, this improved to 37 mm Hg when MRI stroke volume measurements were used to calculate AVA, much closer to the recommended threshold although still discrepant.

Multiple previous studies have shown that a third of patients with moderate and severe aortic stenosis have discordant disease severity according to their AVA and MPG values. Interest has surrounded this group because of its ubiquity and the uncertainty in the outcome associated with these patients. Although some studies have suggested that patients with small-area low-gradient aortic stenosis have a prognosis similar to those with moderate disease, others have indicated the exact opposite and that their outcomes are more akin to those with severe disease.[4,6,7,21]

In the final part of the study, we investigated whether the underestimation of the AVA using echocardiography and inconsistencies in the guideline thresholds might explain the ubiquity of patients with small-area low-gradient aortic stenosis and help resolve the true severity of their disease. We demonstrated that correcting for these 2 factors reduced the number of patients with a small-area low-gradient by > 40%. Of the remaining 29 subjects, 3 had low flow due to an impaired ejection fraction and 2 had low flow due to small LV cavity size. The remainder appeared to genuinely sit on the borderline between moderate and severe disease with parameters that were intermediate between those observed in concordant severe and nonsevere groups. Our data would therefore indicate that discordance in the assessment of aortic stenosis severity can be reduced by correcting for AVA underestimation and inconsistent thresholds, but further studies are now needed to investigate the long-term outcomes of patients reclassified using this approach.

Limitations

In this study, assessment of the planimetered $LVOT_{area}$ using MRI was only available in 40 patients. However, this was believed to be a large enough sample size to assess the inaccuracies associated with LVOT diameter measurements and the data are consistent with the large and expanding literature investigating $LVOT_{area}$ measurements for the sizing of transcatheter aortic valve bioprostheses.[9] Moreover, the baseline characteristics were similar between these 40 patients and the entire cohort of patients with aortic stenosis (see the *Baseline Characteristics of the 40 Patients With Planimetered Left Ventricular Outflow Tract Area on Cardiovascular Magnetic Resonance* section of the Supplementary Material). We also used echocardiography to assess aortic valve calcification. Although this provides important prognostic information,[15] computed tomography provides a more sensitive quantification of aortic valve calcification and has recently been shown to provide differentiation as to the true severity of patients with small-area low-gradient aortic stenosis.[2,22] Phase contrast MRI is an alternate method to estimate stroke volume, but this technique is associated with its own problems, particularly in patients with aortic stenosis in whom complex aortic flow in the ascending aorta can result in measurement inaccuracy. This is a particular problem at 3T. However, in an exploratory analysis, we demonstrated excellent correlation and agreement between phase contrast and volumetric stroke volume on MRI (see the *Comparison of Doppler, MRI Volumetric, and Phase Contrast Stroke Volume Estimation* section of the Supplementary Material). Finally, we were not able to perform echocardiography and MRI on the same day because many of our elderly patients could not tolerate both procedures at the same visit. However, no patient experienced any cardiac events or changes in medications between the 2 scans and after correcting for inaccuracies in the $LVOT_{area}$, an excellent agreement was observed between MRI and echocardiography-derived stroke volumes. This would argue against any significant variability in stroke volumes between the scans.

Conclusions

Echocardiography underestimated the AVA because of an underestimation of the $LVOT_{area}$ and stroke volume, compared with MRI. These factors, along with inconsistent AVA and MPG cutoffs in the current guidelines, account

for > 40% of patients with discordant small-area low-gradient aortic stenosis.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at http://dx.doi.org/10.1016/j.cjca.2014.04.021.

ECHOCARDIOGRAPHY UNDERESTIMATES STROKE VOLUME AND AORTIC VALVE AREA: IMPLICATIONS FOR PATIENTS WITH SMALL-AREA LOW-GRADIENT AORTIC STENOSIS

ONLINE SUPPLEMENTAL DATA

TABLE S1. CHARACTERISTICS OF PATIENTS WITH AORTIC STENOSIS CLASSIFIED BASED ON AORTIC VALVE AREA ESTIMATED USING DOPPLER-DERIVED STROKE VOLUME

	Non-severe (n=44)	Small-area low-gradient (n=56)	Severe (n=28)	P value
Clinical Characteristics				
Age, years	65±13	72±10	68±11	0.02[a]
Males, n (%)	32 (72)	34 (61)	19 (68)	0.44
Height, cm	169±9	163±8	168±8	<0.01[a,b]
Body mass index, kg/m^2	29±5	29±5	27±4	0.13
Body surface area, m^2	1.9±0.2	1.8±0.2	1.9±0.2	0.07
Hypertension, n (%)	27 (61)	40 (71)	16 (57)	0.36
Diabetes Mellitus, n (%)	9 (20)	6 (11)	3 (11)	0.32
Coronary artery disease, n (%)	14 (32)	15 (27)	12 (43)	0.33
Atrial fibrillation, n (%)	0	3 (5)	0	-
Systolic blood pressure, mmHg	147±20	154±20	147±22	0.19
Echocardiography				
Left ventricular outflow tract (LVOT) diameter, cm	2.19±0.21	1.96±0.19	2.08±0.24	<0.01[a,b]
LVOT cross-sectional area, cm^2	3.79±0.75	3.05±0.57	3.43±0.78	<0.01[a]
LVOT velocity time integral, cm	24.5±4.2	23.0±4.5	22.7±4.3	0.15
Doppler stroke volume, mL	92±18	70±13	78±19	<0.01[a,c]
Doppler stroke volume (indexed), mL/m^2	48±10	38±7	42±10	<0.01[a,c]
Aortic valve area, cm^2	1.38±0.38	0.79±0.15	0.69±0.17	<0.01[a,c]
Aortic valve area (indexed), cm^2/m^2	0.72±0.20	0.43±0.08	0.37±0.09	<0.01[a,c]
Mean pressure gradient, mmHg	20±8	29±9	54±17	<0.01[a,b,c]
Peak aortic velocity, m/s	3.0±0.5	3.7±0.5	4.8±0.6	<0.01 [a,b,c]
Dimensionless index	0.36±0.09	0.26±0.05	0.20±0.04	<0.01 [a,b,c]
Aortic valve calcium score	3 [2,3]	3 [3,4]	4 [4,4]	<0.01 [a,b,c]
Valvuloarterial impedance, mmHg•ml^{-1}•m^{-2}	3.6±0.8	4.9±1.1	5.0±1.2	<0.01[a,c]

End-diastolic volume, mL[¶]	94±21	82±26	83±23	0.03[a]
End-diastolic volume (indexed), mL/m^2 [¶]	49±10	45±13	44±12	0.15
End-systolic volume, mL[¶]	42±12	36±14	35±14	0.04
End-systolic volume (indexed), mL/m^2 [¶]	22±6	19±7	19±7	0.11
Stroke volume, mL[¶]	53±12	46±13	48±13	0.04[a]
Stroke volume (indexed), mL/m^2 [¶]	28±6	25±7	26±7	0.25
Ejection fraction, %[¶]	56±7	57±7	59±8	0.40
Mild mitral regurgitation, n (%)	3 (7)	9 (16)	7 (25)	0.10
Mild aortic regurgitation, n (%)	18 (41)	27 (48)	12 (43)	0.24
Cardiovascular Magnetic Resonance				
LVOT cross-sectional area, cm^2 [‡]	4.22±1.21 (n=13)	3.58±0.83 (n=14)	4.53±1.24 (n=12)	0.09
End-diastolic volume, mL	142±30	126±25	139±40	0.03[a]
End-diastolic volume (indexed) (EDVi), mL/m^2	74±13	69±13	74±19	0.17
End-systolic volume, mL	47±17	43±15	47±20	0.38
End-systolic volume (indexed), mL/m^2	25±8	23±8	25±10	0.75
Stroke volume, mL	95±19	83±16	92±26	<0.01[a]
Stroke volume (indexed), mL/m^2	49±8	45±8	49±12	0.08
Ejection fraction, %	67±7	66±7	67±7	0.84
Left ventricular mass (indexed) (LVMi), g/m^2	85±18	85±21	99±25	<0.01[b,c]
LVMi/EDVi, g/mL	1.17±0.23	1.24±0.24	1.38±0.28	<0.01[b,c]

[¶] Estimated using the Teichholz formula
[‡] Planimetered left ventricular outflow tract area was performed in 40 patients. One patient was classified with large-area high-gradient aortic stenosis

[a] P<0.05 between non-severe and small-area low-gradient aortic stenosis
[b] P<0.05 between small-area low-gradient and severe aortic stenosis
[c] P<0.05 between non-severe and severe aortic stenosis

TABLE S2. CHARACTERISTICS OF PATIENTS WITH DISCORDANT SMALL-AREA LOW-GRADIENT AORTIC STENOSIS AFTER CORRECTION FOR STROKE VOLUME UNDERESTIMATION AND INCONSISTENT THRESHOLDS

	Non-severe (n=61)	Small-area low-gradient (n=29)	Severe (n=33)	P value
Clinical Characteristics				
Age, years	66±13	73±9	72±9	<0.01[a,c]
Male, n (%)	44 (72)	15 (52)	20 (61)	0.15
Height, cm	168±9	161±9	165±8	<0.01[a]
Body mass index, kg/m^2	29±5	30±5	27±3	0.09
Body surface area, m^2	1.9±0.2	1.8±0.2	1.8±0.2	0.02
Hypertension, n (%)	38 (62)	21 (72)	22 (67)	0.64
Diabetes mellitus, n (%)	11 (18)	3 (10)	4 (12)	0.56
Coronary artery disease, n (%)	18 (30)	7 (24)	17 (52)	0.04
Atrial fibrillation, n (%)	-	3 (10)	-	-
Systolic blood pressure, mmHg	149±21	151±22	151±23	0.91
Echocardiography				
Left ventricular outflow tract (LVOT) diameter, cm	2.14±0.21	1.94±0.21	2.02±0.25	<0.01[a,c]
LVOT cross-sectional area, cm^2	3.64±0.73	3.01±0.63	3.28±0.82	<0.01[a]
LVOT velocity time integral, cm	23.6±4.2	23.3±5.1	23.7±4.4	0.93
Doppler stroke volume, mL	86±19	69±14	77±20	<0.01[a]
Doppler stroke volume (indexed), mL/m^2	45±10	38±8	42±10	<0.01[a]
Aortic valve area, cm^2	1.24±0.41	0.76±0.16	0.71±0.19	<0.01[a,c]
Aortic valve area (indexed), cm^2/m^2	0.65±0.22	0.42±0.10	0.39±0.10	<0.01 [a,c]
Mean pressure gradient, mmHg	21±8	30±5	55±24	<0.01[a,b,c]
Peak aortic velocity, m/s	3.1±0.6	3.7±0.3	4.8±0.9	<0.01[a,b,c]
Dimensionless index	0.34±0.09	0.26±0.05	0.21±0.05	<0.01[a,b,c]
Aortic valve calcium score	3 [2,3]	4 [3,4]	4 [4,4]	<0.01[a,b,c]
Valvuloarterial impedance, mmHg•ml^{-1}•m^{-2}	4.0±1.0	4.8±1.2	4.9±1.3	<0.01[a,c]

End-diastolic volume, mL[¶]	93±25	77±25	83±24	<0.01[a]
End-diastolic volume (indexed), mL/m^2 [¶]	49±12	42±13	45±13	0.09
End-systolic volume, mL[¶]	42±14	31±13	35±14	<0.01[a]
End-systolic volume (indexed), mL/m^2 [¶]	22±7	17±7	20±7	0.02[a]
Stroke volume, mL [¶]	52±13	45±13	45±11	0.02
Stroke volume (indexed), mL/m^2 [¶]	27±6	25±7	25±7	0.27
Ejection fraction, % [¶]	56±6	60±7	57±9	0.05[a]
Mild mitral regurgitation, n (%)	7 (11)	4 (14)	7 (21)	0.44
Mild aortic regurgitation, n (%)	24 (39)	16 (56)	16 (48)	0.34
Cardiovascular Magnetic Resonance				
LVOT cross-sectional area, cm^2 [‡]	4.30±1.05 (n=16)	3.40±0.90 (n=8)	4.10±1.36 (n=12)	0.20
End-diastolic volume, mL	142±28	117±18	127±35	<0.01[a,c]
End-diastolic volume (indexed) (EDVi), mL/m^2	74±14	65±9	70±17	0.01[a]
End-systolic volume, mL	47±16	39±10	45±22	0.12
End-systolic volume (indexed), mL/m^2	25±8	22±5	25±11	0.28
Stroke volume, mL	95±18	78±13	83±21	<0.01[a,c]
Stroke volume (indexed), mL/m^2	50±9	43±7	45±10	<0.01[a]
Ejection fraction, %	67±7	67±6	66±9	0.63
Left ventricular mass (indexed) (LVMi), g/m^2	86±21	81±18	93±20	0.06
LVMi/EDVi, g/mL	1.18±0.22	1.25±0.23	1.37±0.30	<0.01[c]

[¶] Estimated using the Teichholz formula
[‡] Planimetered left ventricular outflow tract area was performed in 40 patients. Four patients were classified with large-area high-gradient aortic stenosis

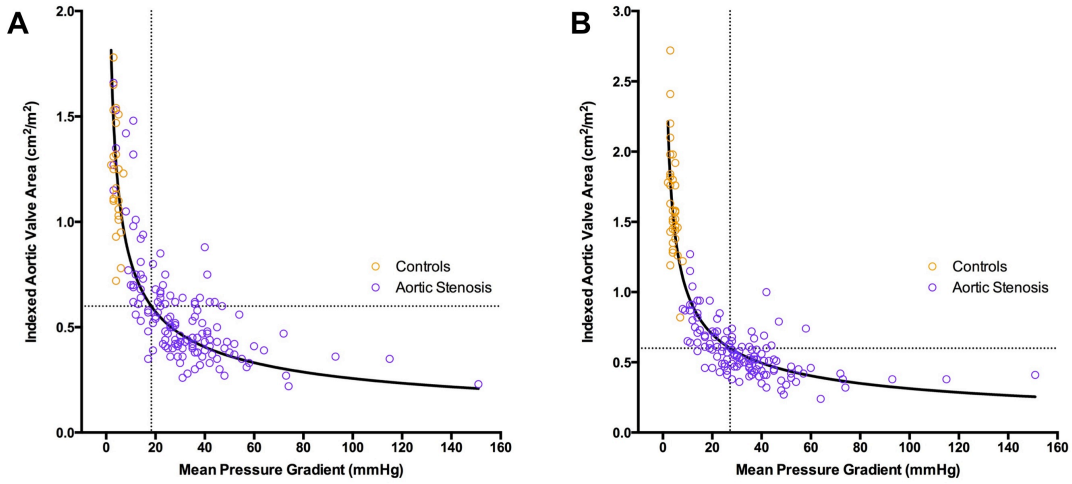
[a] P<0.05 between non-severe and small-area low-gradient aortic stenosis
[b] P<0.05 between small-area low-gradient and severe aortic stenosis
[c] P<0.05 between non-severe and severe aortic stenosis

EVALUATION OF AORTIC STENOSIS CLASSIFICATION USING INDEXED AORTIC VALVE AREA

We investigated thresholds of severe aortic stenosis using indexed aortic valve area, and the effects on classification using an indexed aortic valve area of 0.6 cm^2/m^2, and mean pressure gradient of 40 mmHg.

Using Doppler stroke volume, an indexed aortic valve area of 0.6 cm^2/m^2 corresponded to a mean pressure of 18 mmHg (Figure S1A) while an indexed aortic valve area of 0.6 cm^2/m^2 corresponded to a mean pressure gradient of 27 mmHg with MRI-derived stroke volume (Figure S1B). The use of indexed aortic valve area did not reduce the number of patients with discordant small-area low-gradient aortic stenosis with either Doppler stroke volume [61 patients (46%) compared with the 56 patients (42%) using non-indexed aortic valve area] or MRI-derived stroke volumes [52 patients (39%) compared with the 36 patients (27%) using non-indexed aortic valve area]. These results are also consistent with recent studies (Minners et al., Heart 2010; Jander et al., Heart 2013).

FIGURE S1



EVALUATION OF USING THE DIMENSIONLESS INDEX IN AORTIC STENOSIS CLASSIFICATION

Using a dimensionless index (DI) threshold of <0.25 and mean pressure gradient of <40mmHg, 26 patients were classified with discordant low-DI low-gradient aortic stenosis (20%). This appears to support our conclusion that discordant small-area low-gradient aortic stenosis is largely influenced by left ventricular outflow tract area ($LVOT_{area}$) estimation.

However, this result has to be interpreted with caution. The use of DI has major limitations precisely because it does not take into account the left ventricular outflow tract area ($LVOT_{area}$), which is the key factor to consider when determining the severity of aortic stenosis (Michelena et al., Heart 2012; Baumgartner et al., JASE 2009). This is perhaps best illustrated with an example:

$$\textbf{Aortic valve area} = \textbf{LVOTd}^2 \times 0.785 \times \textbf{DI};\ \textbf{DI} = \textbf{LVOT}_{\textbf{VTI}}/\textbf{AV}_{\textbf{VTI}}$$

In a patient with a LVOT diameter (LVOTd) of 2.0 cm and DI of 0.25 (severe aortic stenosis), this would translate to an aortic valve area of 0.79 cm^2 (severe aortic stenosis). However, in another patient with LVOTd of 2.5 cm and the same DI of 0.25, this increases the aortic valve area to 1.23 cm^2 (moderate aortic stenosis). This example illustrates that a DI threshold of 0.25 may not be appropriate in all patients: in patients with large LVOT, a smaller DI threshold for severe disease may be needed (Michelena et al., Heart 2012). Indeed, amongst the 26 patients with discordant low-DI low-gradient aortic stenosis, 9 patients (35%) had an aortic valve area > 1.0cm^2 and they had larger mean LVOTd (measured on 2D echocardiography) compared to the other 17 patients (2.2±0.2 versus 1.9±0.2 cm, P=0.03).

Accurate estimation of the $LVOT_{area}$ is therefore critical in assessing aortic stenosis severity. Our study highlights the limitations that echocardiography has in making such measurements and how improved stroke volume estimation can have important implications in the grading of aortic stenosis.

BASELINE CHARACTERISTICS OF THE 40 PATIENTS WITH PLANIMETERED LEFT VENTRICULAR OUTFLOW TRACT AREA ON CARDIOVASCULAR MAGNETIC RESONANCE

In this study, 40 patients with mild to severe aortic stenosis were randomly selected and planimetry of the left ventricular outflow tract area ($LVOT_{area}$) was performed on cardiovascular magnetic resonance. The purpose is to investigate the effects of accurate $LVOT_{area}$ measurement on stroke volume estimation.

The characteristics of these 40 patients were similar to the entire cohort of patients with aortic stenosis (Table S3).

TABLE S3

Characteristics	Subgroup (n=40)	All patients with aortic stenosis (n=133)	P value
Age, years	68±12	69±12	0.64
Males, n (%)	27 (68)	83 (63)	0.56
Body surface index, m^2	1.9±0.2	1.9±0.2	1.00
Systolic blood pressure, mmHg	151±21	150±21	0.79
Heart rate, per min	64±10	64±11	1.00
Mean pressure gradient, mmHg	37±24	32±16	0.13
Peak aortic jet velocity, m/s	4.0±1.1	3.7±0.8	0.06
Aortic valve area, cm^2	1.0±0.3	1.0±0.4	1.00
Indexed end-diastolic volume (EDV), mL/m^2	75±21	72±16	0.34
Indexed end-systolic volume, mL/m^2	25±12	24±9	0.57
Indexed stroke volume, mL/m^2	50±12	48±10	0.29
Ejection fraction, %	67±8	67±7	1.00
Indexed left ventricular mass (LVM), g/m^2	95±28	88±21	0.09
LV mass/EDV, g/mL	1.29±0.28	1.25±0.26	0.40

COMPARISON OF DOPPLER, MRI VOLUMETRIC AND PHASE CONTRAST STROKE VOLUME ESTIMATION

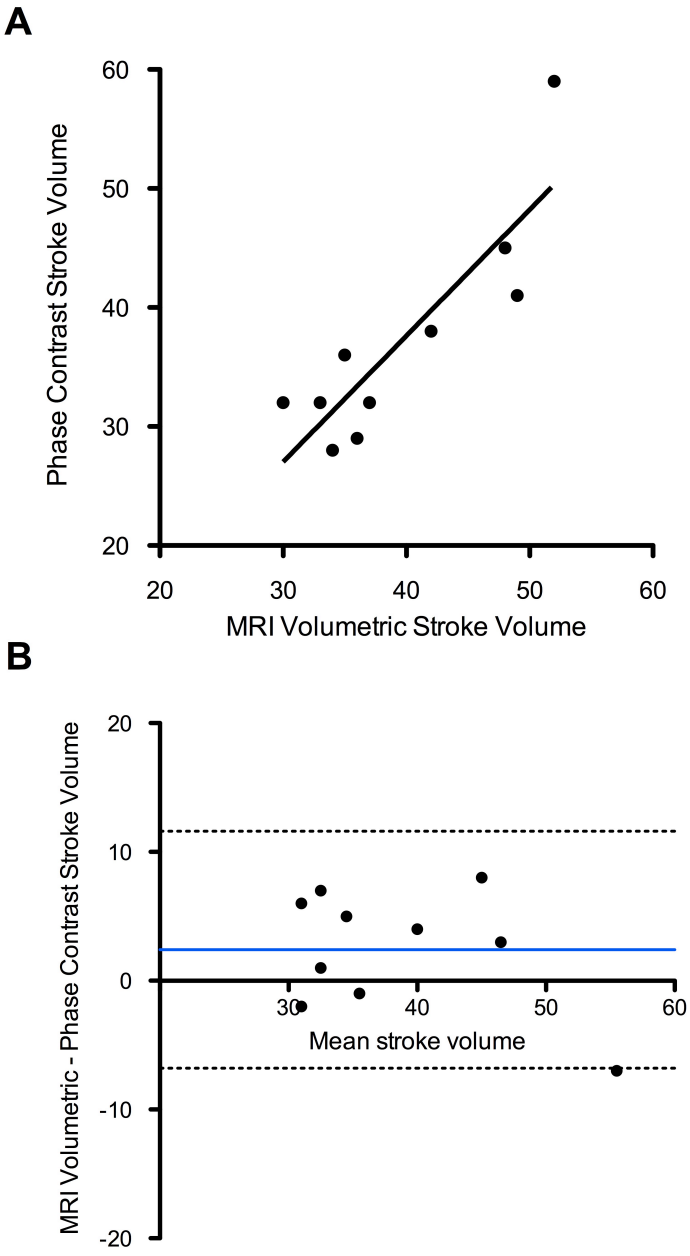
An exploratory analysis was performed in 10 patients with aortic stenosis to compare Doppler, MRI volumetric (cine) and phase contrast stroke volume. Through plane phase contrast velocity mapping was positioned orthogonal to the ascending aorta at the level of the bifurcation of the main pulmonary artery. An initial velocity encoding level of 100 cm/s was selected and increased in increments of 50 cm/s if aliasing occurs. The total forward flow during systole is computed using the Argus software (Siemens AG Healthcare Sector, Erlangen, Germany).

The results are shown in Table S4. In these 10 patients, there was no correlation between Doppler indexed stroke volume and MRI-derived indexed stroke volume (r=0.32; P=0.37) and between Doppler indexed stroke volume and MRI phase contrast indexed stroke volume (r=0.20; P=0.58). On the other hand, MRI-derived stroke volume and MRI phase contrast demonstrated excellent correlation (r=0.87; P=0.001; Figure S2A) and agreement (2.4mL/m^2; 95% CI -6.8 to 11.6 mL/m^2; Figure S2B).

TABLE S4

Patient S/N	Echocardiographic indexed stroke volume (mL/m^2)	MRI-derived indexed stroke volume (mL/m^2)	Phase contrast indexed flow volume (mL/m^2)	Aortic stenosis severity
#1	34	49	41	Severe
#2	33	33	32	Severe
#3	45	34	28	Moderate
#4	35	35	36	Moderate
#5	46	37	32	Severe
#6	54	36	29	Severe
#7	50	48	45	Mild
#8	45	42	38	Moderate
#9	36	30	32	Severe
#10	49	52	59	Mild

FIGURE S2


A
Phase Contrast Stroke Volume
60
50
40
30
20
20
30
40
50
60
MRI Volumetric Stroke Volume
B
MRI Volumetric - Phase Contrast Stroke Volume
20
10
0
-10
-20
30
40
50
60
Mean stroke volume

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CLINICAL RESEARCH
Valvular heart disease

High-sensitivity troponin I concentrations are a marker of an advanced hypertrophic response and adverse outcomes in patients with aortic stenosis

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Aims High-sensitivity cardiac troponin I (cTnI) assays hold promise in detecting the transition from hypertrophy to heart failure in aortic stenosis. We sought to investigate the mechanism for troponin release in patients with aortic stenosis and whether plasma cTnI concentrations are associated with long-term outcome.

Methods and results Plasma cTnI concentrations were measured in two patient cohorts using a high-sensitivity assay. First, in the Mechanism Cohort, 122 patients with aortic stenosis (median age 71, 67% male, aortic valve area 1.0 $\pm$ 0.4 cm^2) underwent cardiovascular magnetic resonance and echocardiography to assess left ventricular (LV) myocardial mass, function, and fibrosis. The indexed LV mass and measures of replacement fibrosis (late gadolinium enhancement) were associated with cTnI concentrations independent of age, sex, coronary artery disease, aortic stenosis severity, and diastolic function. In the separate Outcome Cohort, 131 patients originally recruited into the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact of REgression (SALTIRE) study, had long-term follow-up for the occurrence of aortic valve replacement (AVR) and cardiovascular deaths. Over a median follow-up of 10.6 years (1178 patient-years), 24 patients died from a cardiovascular cause and 60 patients had an AVR. Plasma cTnI concentrations were associated with AVR or cardiovascular death HR 1.77 (95% CI, 1.22 to 2.55) independent of age, sex, systolic ejection fraction, and aortic stenosis severity.

Conclusions In patients with aortic stenosis, plasma cTnI concentration is associated with advanced hypertrophy and replacement myocardial fibrosis as well as AVR or cardiovascular death.

Keywords Aortic stenosis • High-sensitivity troponin • Left ventricular hypertrophy • Myocardial fibrosis • Cardiac magnetic resonance

Introduction

Aortic stenosis is the commonest form of valvular heart disease in the western world, and its prevalence is expected to double in the next two decades.[1] Current guidelines advocate aortic valve replacement (AVR) in patients with symptoms and severe valve narrowing.[2,3] However, there is a poor correlation between the severity of stenosis and symptom onset making the management of asymptomatic

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patients controversial.[2] This apparent discrepancy might in part be explained by heterogeneity in the hypertrophic response to aortic stenosis, which itself is an independent marker of an adverse prognosis.[4–6]

Hypertrophy occurs in response to the increased afterload imposed by aortic valve narrowing on the left ventricle. Initially this restores wall stress and maintains cardiac performance, but decompensation ultimately ensues and patients develop symptoms, adverse events, and the need for surgery. The transition from hypertrophy to heart failure is characterized by progressive cardiomyocyte death and replacement fibrosis.[7] Myocardial fibrosis can be detected using two cardiovascular magnetic resonance (CMR) techniques: late gadolinium enhancement (replacement fibrosis) and T1 mapping (diffuse interstitial fibrosis) with data indicating that the former provides useful prognostic information.[5,8] However, to date, a marker of myocyte cell death has been lacking.

Cardiac troponin is a structural protein present in cardiac muscle, with plasma troponin concentrations considered a highly specific marker for myocardial injury.[9] Recent advances in assay technology have greatly improved sensitivity, now allowing quantification of troponin with a high degree of precision at extremely low plasma concentrations.[10]

In this study, we hypothesized that detection of myocardial injury by high-sensitivity troponin assays may provide an early indicator of left ventricular (LV) decompensation and be associated with future adverse events in patients with aortic stenosis.

Methods

Two cohorts of stable patients with aortic stenosis were recruited from cardiology outpatient clinics across three centres in Southeast Scotland. First, we determined the association between plasma cardiac troponin I (cTnI) concentrations and LV functional and structural abnormalities on cardiac magnetic resonance (Mechanism Cohort), and second, we examined the prognostic role of plasma cTnI concentrations in patients with aortic stenosis (Outcome Cohort). The study was conducted in accordance with the Declaration of Helsinki and approved by the local research ethics committee. Written informed consent was obtained from all participants.

Patient populations

Mechanism Cohort

Patients with mild to severe aortic stenosis were recruited prospectively. We excluded patients who had other significant (moderate or severe) valvular heart disease or cardiomyopathies (acquired or inherited). Presence of coronary artery disease was defined by previous infarction, clinical symptoms of angina (in those with mild or moderate aortic stenosis), evidence of myocardial ischaemia, or >50% luminal stenosis in a major epicardial vessel. In addition, thirteen age- and sex-matched healthy volunteers without clinically significant heart disease were recruited from the local community.

Outcome Cohort

The Outcome Cohort comprised patients recruited into the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact of REgression (SALTIRE) study. The study design and inclusion and exclusion criteria have been described previously.[11] In brief, from March 2001 to April 2002, 155 patients with asymptomatic moderate to severe aortic stenosis were randomly assigned to receive either atorvastatin 80 mg or placebo once daily. Patients were excluded if they were already on a statin, if AVR was planned, or if they had moderate or severe LV systolic impairment. Only patients with plasma samples available for cTnI analysis were included in the present analysis.

Blood sampling and analysis

In the Mechanism Cohort, brain natriuretic peptide (BNP) concentration was analysed with the Triage BNP assay (Biosite, Inc., San Diego, CA, USA). The inter-assay coefficient of variation was 10% at 28.8 pg/mL, with a detection range of 5–1300 pg/mL.[12] In the Outcome Cohort, N-terminal proBNP (NT-proBNP) concentration was measured using the Elecsys 2010 analyzer (Roche Diagnostics Ltd, Lewes, UK). This assay has <0.001% cross-reactivity with bioactive BNP, and the inter-assay coefficients of variation range from 0.9 to 5.5%.[13]

Plasma cTnI concentrations were determined by the ARCHITECT *STAT* high-sensitivity cTnI assay (Abbott Laboratories, Abbott Park, Il, USA) in both cohorts. The lower limit of detection of this assay is 1.2 ng/L;[10] the 99th percentile from a healthy reference population is 26 ng/L.[14] Our inter-assay coefficient of variation is 10% at 6 ng/L (see Supplementary material online).

Echocardiography

All participants underwent a comprehensive echocardiographic assessment to determine the severity of aortic stenosis. Peak aortic jet velocity and mean pressure gradient were measured by velocity time integral spectral Doppler, and the aortic valve area derived using the continuity equation. The severity of aortic stenosis was assessed and classified according to the European Association of Echocardiography/American Society of Echocardiology guidelines.[15] Trans-mitral early (E) and late diastolic velocities and deceleration time of early filling velocity were measured at the tips of the mitral valve leaflets using pulse-wave Doppler. Early (e′) diastolic velocities of the medial and lateral mitral annulus were measured using pulse-wave tissue Doppler imaging. Diastolic function was determined using the E/e′ ratio.

Cardiovascular magnetic resonance in the Mechanism Cohort

Cardiovascular magnetic resonance was performed using a 3T scanner (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany). Short-axis cine images were obtained using a balanced steady-state free precession sequence from the mitral valve annulus to the apex (8-mm parallel slices with 2 mm spacing) for the assessment of LV function and volumes. LV volumes, mass, and ejection fraction were assessed using dedicated software (Argus Ventricular Function, Siemens AG Healthcare Sector, Erlangen, Germany), and values were indexed to body surface area.

Focal myocardial fibrosis was assessed using the late gadolinium enhancement (LGE) technique, performed 10–15 min following 0.1 mmol/kg of gadobutrol (Gadovist, Bayer Pharma AG, Germany). Two approaches were used: an inversion-recovery fast gradient echo sequence and a phase-sensitive inversion-recovery sequence (performed in two phase-encoding directions for the exclusion of artefact). The inversion time was optimized to achieve satisfactory nulling of the myocardium. Assessment for the presence of mid-wall LGE was determined visually and independently by two experienced operators. The extent of mid-wall LGE was quantified with QMASS software (Medis Medical Imaging Systems, Leiden, the Netherlands) using a signal intensity threshold of >2 standard deviations above the mean value in an adjacent normal region of myocardium. Areas of inversion artefact, or contamination by blood pool or epicardial fat, were excluded.

Myocardial T1 mapping was performed to investigate diffuse myocardial fibrosis using the Modified Look-Locker Inversion-recovery sequence (flip angle 35°; minimum TI 100 ms; TI increment of 80 ms; time delay of 150 ms with a heartbeat acquisition scheme of 3–3–5).[16] We have previously described a standardized approach for the analysis of myocardial extracellular volume fraction (ECV) in patients with aortic stenosis, demonstrating that it offers improved reproducibility (± 3%) and the ability to identify disease states compared with other T1 mapping techniques.[17] In brief, regions of interest were drawn around the myocardium on short-axis pre-contrast motion-corrected myocardial T1 maps and then applied to corresponding 20-min post-contrast maps with minor adjustments made to avoid partial volume effects and artefact (OsiriX version 4.1.1, Geneva, Switzerland). Extracellular volume fraction values were calculated according to: $ECV = [\Delta R1_{myocardium}/\Delta R1_{blood-pool}] \times$ [1-haematocrit], where $\Delta R1 =$ (1/post-contrast T1-1/pre-contrast T1). Haematocrit was determined at the time of CMR.

Computed tomography in the Outcome Cohort

Computed tomography calcium scoring of the coronary arteries and aortic valve was performed on ECG-gated non-contrast scans using a double helix scanner (Twin II Flash, Philips Medical Systems). All images were analysed by a single operator using the Picker Cardiac Scoring software as previously described.[11]

Follow-up in the Outcome Cohort

Clinical outcomes were obtained and adjudicated by two independent investigators blinded to plasma cTnI and BNP concentrations. All in-hospital and community deaths were captured in a comprehensive national database: the General Register of Scotland. Cardiovascular death was based on the cause of death stated on the death certificate. We defined cardiovascular death as death due to myocardial infarction, sudden cardiac death, heart failure, stroke, death due to cardiovascular procedures, and death due to other cardiovascular causes. Each death was classified as cardiac or non-cardiac by two independent investigators and any discrepancy resolved by consensus. All events were confirmed by independent review of each patient's electronic healthcare record where available. Surgical AVR (no patients underwent transcatheter aortic valve implantation in the follow-up period) was determined from individual patient medical records. All patients in the Outcome Cohort were managed in the tertiary cardiac centre, where patients are reviewed at a multi-disciplinary meeting prior to undergoing cardiac surgery. Only patients with established indications were referred for AVR according to the European Society of Cardiology recommendations.[2,18]

Statistical analysis

Baseline characteristics are reported as percentages for categorical variables, mean ± standard deviation or median [interquartile range], as appropriate. We used one-way analysis-of-variance to compare continuous parametric data and the Kruskall–Wallis test for non-parametric data. Chi-square tests were used for categorical baseline characteristics. Analyses were performed in R version 2.15.2 (Vienna, Austria) and SPSS Version 20.0.0 (Armonk, NY, USA: IBM Corp). Statistical significance was taken as a two-sided $P < 0.05$.

Mechanism Cohort

We assessed the association of plasma cTnI concentrations with measures of aortic stenosis and ventricular remodelling using univariate and multivariable linear regression models. Plasma cTnI concentrations were log-transformed as this variable was highly skewed.

Outcome Cohort

Kaplan–Meier analysis was performed across tertiles of cTnI concentrations. To accommodate competing risks, the association between time to AVR or cardiovascular deaths and plasma cTnI concentrations (log-transformed [base 2]) was modelled as a composite endpoint in Cox proportional hazard models.

Furthermore, we examined whether relative change in cTnI concentrations at 1 year (cTnI at 1 year—baseline cTnI, both log-transformed) was associated with increased odds of an event at 3-year and 5-year follow-up independent of baseline cTnI concentrations (results in Supplementary material online).

Results

We recruited 122 patients into the Mechanism Cohort (71 [65–77] years, 67% males, aortic valve area 1.0 ± 0.4 cm^2) and analysed 131 patients in the Outcome Cohort (69 [62–75] years, 70% males, aortic valve area 1.1 ± 0.4 cm^2) (*Tables 1* and *2*). Thirteen healthy volunteers were recruited, who were well matched in terms of age (65 [57–75] years) and sex (62% male) compared with the other groups and did not have any history of diabetes mellitus, hypertension, or coronary artery disease.

Plasma cTnI concentrations above the lower limit of detection of 1.2 ng/L were present in 98% of our patients with aortic stenosis and increased in both cohorts compared with the healthy volunteers (Mechanism Cohort 6.6 [3.8–12.0] ng/L; Outcome Cohort 7.6 [5.7–13.2] ng/L; healthy volunteers 3.2 [1.3–11.0] ng/L). The distribution of plasma cardiac troponin I concentrations was skewed in a similar pattern across the two cohorts (see Supplementary material online). There were 10 patients (8.1%) in the Mechanism Cohort and 10 patients (7.6%) in the Outcome Cohort with plasma cTnI concentrations of >26 ng/L (the 99th percentile derived from the healthy reference population). There was no difference in renal function across tertiles of cTnI in patients with aortic stenosis.

Mechanism for increased cardiac troponin I concentrations

In the Mechanism Cohort, patients with aortic stenosis had an increased LV mass index compared with healthy controls, although there was no difference in LV volumes or ejection fraction (*Table 1*). Furthermore, these patients had higher ECV values (27.7 ± 2.5 vs. 25.9 ± 1.6%, $P = 0.01$), and 35 patients (28%) had a mid-wall pattern of LGE: an observation not seen among the healthy volunteers (*Figure 1*).

Plasma cTnI concentrations correlated with LV mass index, independent of coronary artery disease status ($r = 0.50$, $P < 0.001$; *Figure 2*). A weaker correlation was also observed between plasma cTnI concentrations and peak aortic jet velocity ($r = 0.32$, $P < 0.001$). Furthermore, patients with aortic stenosis and mid-wall LGE had a two-fold increase in plasma cTnI concentrations compared with those without (9.5 [5.7, 20.3] ng/L vs. 4.3 [3.3, 7.9] ng/L, $P = 0.02$; *Figure 3*).

With univariate analysis, age, mean pressure gradient, mean e′, the LV mass index, and measures of both diffuse and replacement fibrosis

Table 1 Baseline characteristics of patients with aortic stenosis in the Mechanism Cohort

	Healthy volunteers ($n = 13$)	Mechanism Cohort ($n = 122$)	*P*-value
Clinical characteristics			
Age, years	65 [57, 75]	71 [65, 77]	0.13
Male sex, *n* (%)	8 (62)	82 (67)	0.76
Diabetes mellitus, *n* (%)	0	14 (11)	–
Hypertension, *n* (%)	0	78 (63)	–
CAD, *n* (%)	0	41 (33)	–
SBP, mmHg	148 ± 12	149 ± 20	0.35
NYHA class, *n* (%)			
I	13 (100)	63 (52)	–
II	0	35 (28)	
III	0	24 (20)	
Creatinine, μmol/L	69 ± 8	78 ± 17	0.06
Cardiac troponin I concentration, ng/L	3.2 [1.3, 11.0]	6.6 [3.8, 12.0]	0.03
BNP, pg/mL	10.3 [5.6, 18.1]	26.4 [10.6, 53.9]	0.009
Echocardiography			
V_m, m/s	1.4 ± 0.2	3.7 ± 0.9	<0.001
MPG, mmHg	4 ± 1	32 ± 18	<0.001
AVA, cm^2	2.4 ± 0.7	1.0 ± 0.4	<0.001
Valvulo-arterial impedance, $mmHg/mL/m^2$	4.5 ± 1.1	4.5 ± 1.2	0.96
Mean e′, cm/s	8.1 ± 2.7	6.2 ± 1.9	0.001
Mean E/e′	7.9 ± 2.2	14.8 ± 8.1	0.003
Cardiac MRI			
Indexed EDV, mL/m^2	73 ± 13	72 ± 14	0.71
Indexed ESV, mL/m^2	27 ± 7	24 ± 9	0.28
Indexed SV, mL/m^2	46 ± 7	48 ± 9	0.68
Ejection fraction, %	64 ± 3	67 ± 7	0.12
Indexed LVM, g/m^2	70 ± 14	89 ± 22	0.004
LVM/EDV, g/mL	0.96 ± 0.13	1.26 ± 0.28	<0.001
ECV, %	25.9 ± 1.6	27.7 ± 2.5	0.01

CAD, coronary artery disease; SBP, systolic blood pressure; BNP, brain natriuretic peptide; V_m, peak aortic jet velocity; MPG, mean pressure gradient; AVA, aortic valve area; EDV, end diastolic volume; ESV, end systolic volume; LVM, left ventricular mass; ECV, extracellular volume fraction; %LGE, amount of late gadolinium enhancement.

were all associated with plasma cTnI concentrations (*Table 3*; all $P < 0.05$). However, only age, LV mass index, and %LGE were independently associated with plasma cTnI concentrations (Model 1; *Table 3*).

Interestingly, there was no difference in plasma cTnI concentrations between patients with and without coronary artery disease (6.9 [4.0, 13.5] ng/L vs. 6.2 [3.5, 10.0] ng/L, $P = 0.28$). This was supported by data from the Outcome Cohort where no correlation was observed between the coronary calcium scores and plasma cTnI concentrations ($r = -0.03$, $P = 0.71$).

Prognostic value of cardiac troponin I concentrations

Patients in the Outcome Cohort were stratified by tertiles of plasma cTnI concentration (*Table 2*). In comparison with the lowest tertile, patients in the highest tertile were older (70 ± 9 vs. 64 ± 12 years, $P = 0.03$) and had an increased ventricular mass (393 ± 111 g, $P = 0.02$). However, there were no differences in co-morbidity, severity of aortic stenosis, or coronary calcium scores across the tertiles ($P > 0.1$ for all; *Table 2*).

Over a median of 10.6 years of follow-up (1178 patient-years), 60 patients had an AVR, 24 died from a cardiovascular cause, and 47 died from non-cardiovascular causes. Ten-year event-free survival rate for AVR or cardiovascular deaths differed across the tertiles of cTnI concentrations (log rank test for trend, $P = 0.016$, *Figure 4*). Plasma cTnI concentration was associated with an increased risk of AVR or cardiovascular deaths in unadjusted analysis (HR 1.65 per two-fold increment in cTnI concentration; 95% CI, 1.15–2.38, $P = 0.007$) with minimal attenuation in the effect estimate after adjusting for age, sex, and ejection fraction (*Table 4*). Moreover, this association persisted after further adjustment for severity of aortic stenosis (HR 1.77; 95% CI, 1.22–2.35, $P = 0.002$) as well as the coronary artery and aortic valve calcium scores (HR 2.10; 95% CI, 1.22–3.61, $P = 0.007$).

Table 2 Characteristics of patients in the Outcome Cohort by tertiles of troponin I concentrations

	All patients ($n = 131$)	Tertile 1 (≤6.3 ng/L) ($n = 42$)	Tertile 2 (6.4–10.6 ng/L) ($n = 45$)	Tertile 3 (≥10.7 ng/L) ($n = 44$)	*P*-value
Clinical characteristics					
Age, years	67 ± 10	64 ± 12	69 ± 10	70 ± 9	0.03
Male sex, *n* (%)	91 (70)	24 (57)	32 (71)	35 (79)	0.08
Diabetes Mellitus, *n* (%)	4 (3)	1 (2)	1 (2)	2 (5)	–
Hypertension, *n* (%)	66 (50)	18 (43)	22 (49)	26 (59)	0.31
CAD, *n* (%)	22 (16)	6 (14)	7 (16)	9 (21)	0.72
SBP, mmHg	145 ± 20	139 ± 17	148 ± 21	146 ± 19	0.07
NYHA class, *n* (%)					
I	117 (89)	38 (90)	41 (91)	38 (86)	0.53
II	14 (11)	4 (10)	4 (9)	6 (14)	
Creatinine, μmol/L	91 ± 21	86 ± 17	92 ± 20	95 ± 25	0.12
NT-pro-BNP, pg/mL	198.0 [113.5, 530.5]	129.5 [76.3, 228.0]	180.0 [89.0, 416.0]	507.0 [181.5, 1103.0]	0.008
Echocardiography					
V_m, m/s	3.4 ± 0.7	3.4 ± 0.6	3.4 ± 0.6	3.5 ± 0.7	0.45
MPG, mmHg	26 ± 11	25 ± 10	25 ± 10	28 ± 13	0.35
AVA, cm^2	1.1 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	1.0 ± 0.4	0.72
Indexed AVA, cm^2/m^2	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.66
LVM, g	357 ± 107	327 ± 111	350 ± 102	393 ± 100	0.02
Indexed LVM, g/m^2	180 ± 50	165 ± 54	172 ± 49	196 ± 49	0.06
Fractional shortening, %	40 ± 8	42 ± 9	42 ± 8	37 ± 6	0.004
Ejection fraction, %	70 ± 10	72 ± 11	72 ± 9	66 ± 8	0.007
LVH, *n* (%)	109 (95)	34 (81)	36 (80)	39 (89)	0.49
Impaired LVEF <50%, *n* (%)	4 (3)	1 (2)	2 (4)	2 (5)	0.84
Computed tomography					
Coronary calcium score, log AU	1.6 ± 1.3	1.5 ± 1.3	1.5 ± 1.3	1.8 ± 1.1	0.53
Aortic valve calcium score, log AU	3.6 ± 0.6	3.6 ± 0.5	3.6 ± 0.5	3.7 ± 0.8	0.61

CAD, coronary artery disease; SBP, systolic blood pressure; BNP, brain natriuretic peptide; V_m, peak aortic jet velocity; MPG, mean pressure gradient; AVA, aortic valve area; EDV, end diastolic volume; ESV, end systolic volume; LVM, left ventricular mass; LVH, left ventricular hypertrophy, based on ASE/EAE sex-specific criteria; LVEF, left ventricular ejection fraction.

Mechanism and prognosis associated with BNP concentrations

Serum BNP concentrations were higher in patients with aortic stenosis compared with healthy volunteers (26.4 [10.6,53.9] vs. 10.3 [5.6,18.1] ng/mL, $P = 0.009$; *Table 1*). In patients with aortic stenosis, BNP concentrations increased with age, disease severity, diastolic dysfunction, LV mass index myocardial fibrosis, the presence of coronary artery disease, and symptoms (all $P < 0.05$; see Supplementary material online). However, on multivariable analysis, only age was significantly associated with BNP concentrations ($P < 0.001$; see Supplementary material online).

In the Outcome Cohort, NT-proBNP was not associated with AVR or cardiovascular deaths in both unadjusted (HR 1.15 per two-fold increment in NT-proBNP concentration; 95% CI, 0.86–1.53, $P = 0.34$) and adjusted analyses (see Supplementary material online). Importantly, NT-proBNP concentration did not modify the association between troponin and time to AVR or cardiovascular deaths (HR 1.60; 95% CI 1.10–2.34, $P = 0.01$).

Discussion

This is the first dataset to explore mechanisms and outcomes associated with cTnI concentrations using a high-sensitivity assay in patients with aortic stenosis. In more than 250 patients with aortic stenosis, we have demonstrated that levels are detectable in 98% of subjects and increased compared with age- and sex-matched healthy volunteers. Plasma cTnI concentrations were not associated with the presence of co-existent coronary artery disease or the severity of valve narrowing on multivariable analysis. Instead, plasma cTnI concentrations demonstrated a close association with the magnitude of LV hypertrophy and the presence of mid-wall myocardial fibrosis. Moreover, high-sensitivity plasma cTnI concentration showed an independent association with long-term risk of AVR or cardiovascular deaths. We therefore believe that high-sensitivity plasma cTnI concentrations hold potential as an objective marker of LV decompensation in patients with aortic stenosis and as a potential early trigger to AVR.

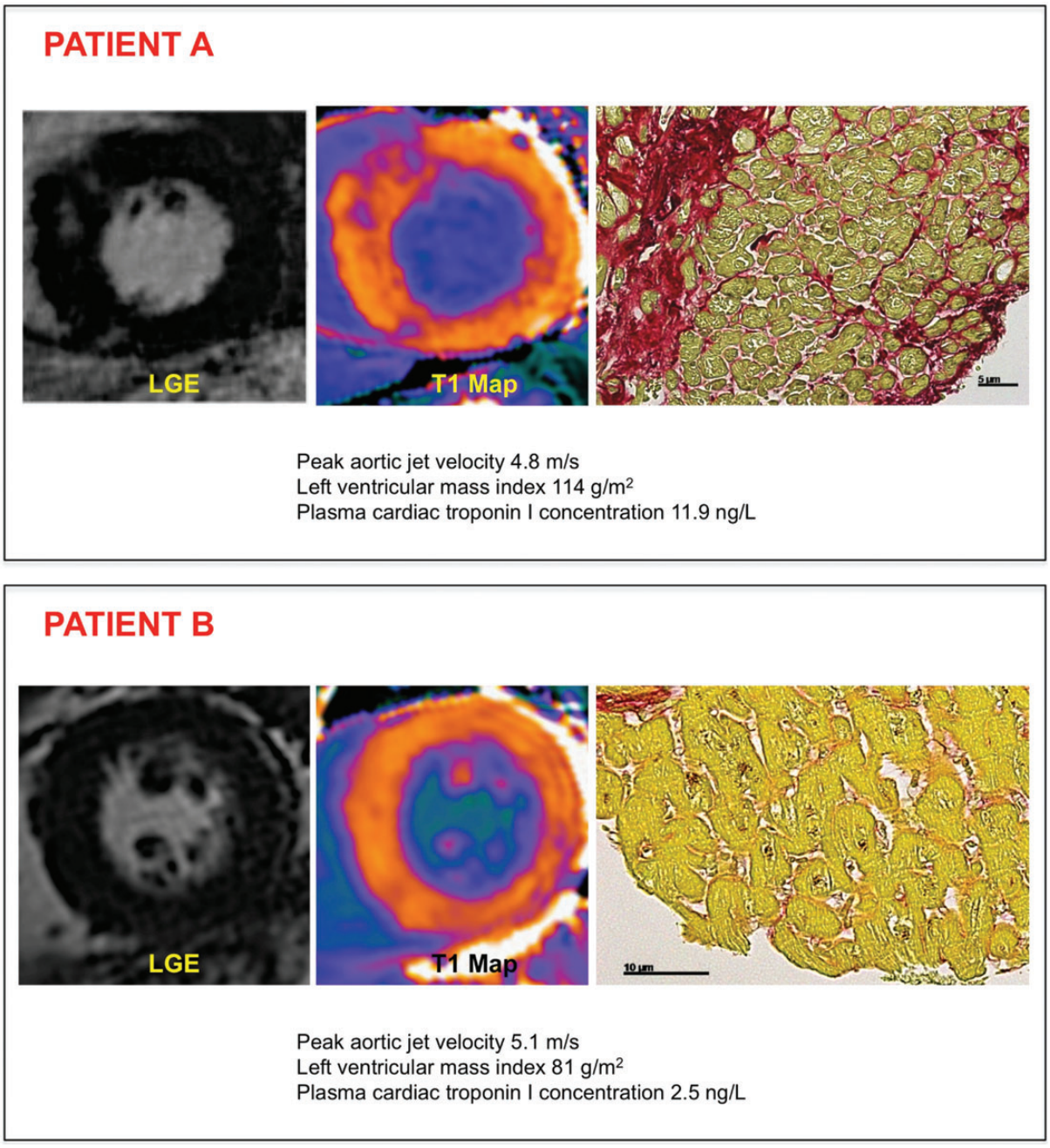

Figure 1 Comparison of two patients with severe aortic stenosis. Both had similar severity of aortic valve narrowing (peak aortic jet velocity in Patient A was 4.8 m/s and Patient B 5.1 m/s) and neither had significant coronary artery disease. However, the high-sensitivity troponin I concentration was more than four-fold higher in Patient A (11.9 ng/L) compared with Patient B (2.5 ng/L), consistent with the more advanced hypertrophic response observed in this patient (left ventricular mass index in Patient A was 114 g/m^2 and Patient B was 81 g/m^2). Furthermore, Patient A had evidence of focal mid-wall fibrosis on late gadolinium imaging (LGE) and myocardial T1 mapping (Patient B did not) and more extensive collagen staining with picrosirius red staining on myocardial biopsy.

Aortic stenosis is defined not only by the development of progressive valve narrowing but also by the LV hypertrophic response that ensues. Whilst this initially restores wall stress, decompensation due to progressive cell death and fibrosis ultimately occurs and patients transition from hypertrophy to heart failure.[4] Because of the associated adverse prognosis, current guidelines recommend surgery in patients with severe stenosis and evidence of such decompensation, detected either on the basis of symptom development or an ejection fraction of $<$50%. Unfortunately, symptoms are often frequently difficult to assess whereas an ejection fraction of $<$50%

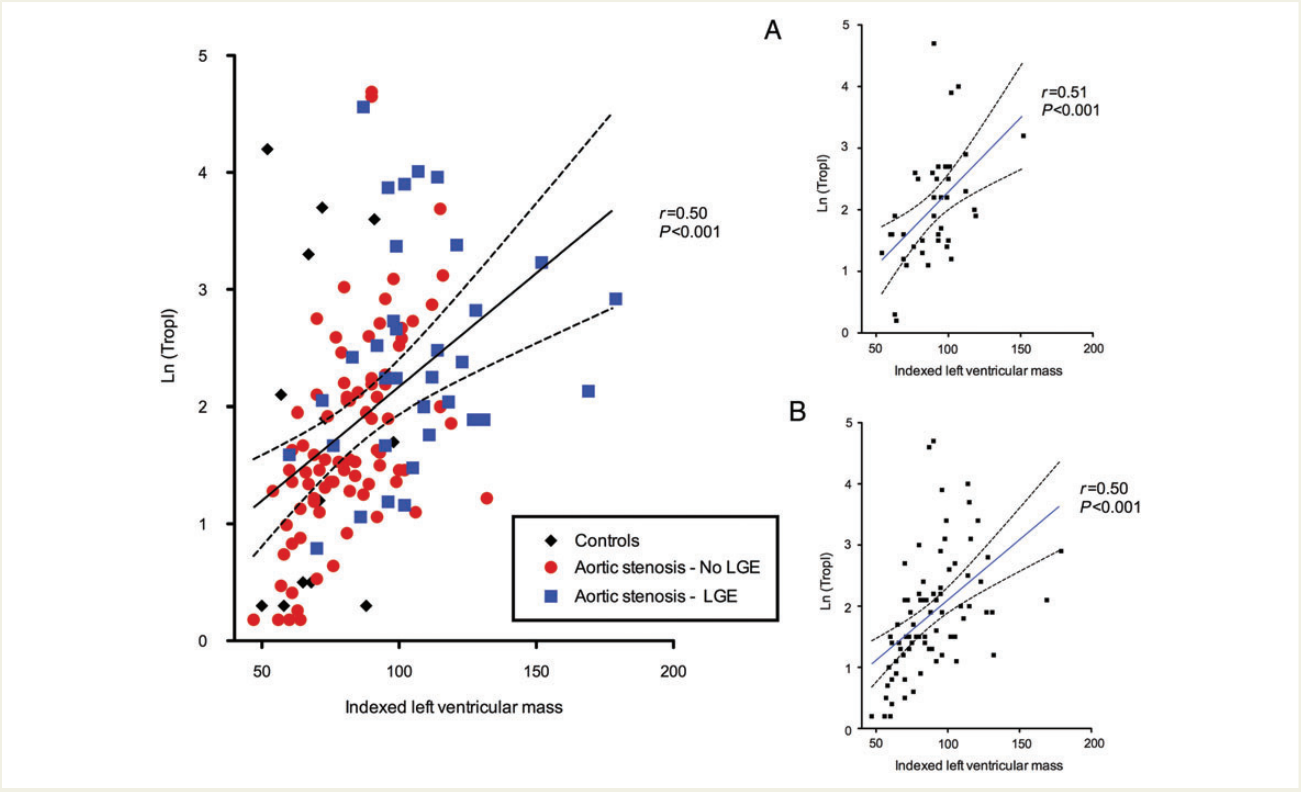

Figure 2 Correlation between indexed left ventricular mass and plasma cardiac troponin I concentrations (log-transformed). Similar correlation was seen in patients with (*A*) and without (*B*) coronary artery disease.

occurs late in the disease process and is often irreversible. There is therefore emerging interest in developing novel, objective biomarkers of decompensation for patients with aortic stenosis. Data from our study suggests that troponin has the potential to be such a marker.

To date elevated cardiac troponin has been considered the *sine qua non* for the diagnosis of myocardial infarction.[19] However, marked improvements in assay sensitivity now allow quantification of plasma cTnI concentrations in the majority of the healthy population.[10] In our study, cTnI was detectable in 98% of patients with aortic stenosis and exceeded the recommended diagnostic threshold for myocardial infarction in 7.9%. Patients with stable coronary disease have been reported to have higher plasma troponin concentrations, with elevated levels being associated with long-term cardiovascular risk.[20] However in our cohort of patients with aortic stenosis, there were no differences in plasma troponin concentrations between those with and without coronary artery disease. Instead plasma troponin concentrations were independently associated with an advanced hypertrophic response and replacement myocardial fibrosis. Indeed, the latter occurred over and above the effects of LV mass, supporting our hypothesis that cTnI release relates to the myocardial injury that accompanies ventricular decompensation and myocardial fibrosis.

The poor prognosis associated with increased troponin concentrations offers further support for this model. At 10 years, more than a half of patients in the highest tertile of plasma cTnI had undergone an AVR or died from cardiovascular disease. Moreover, plasma

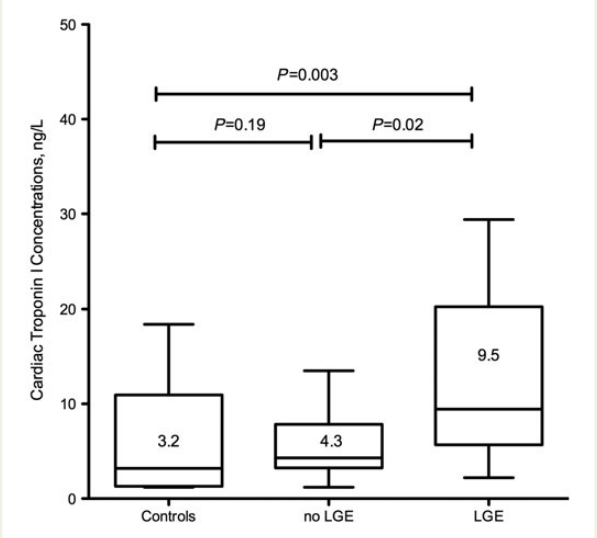

Figure 3 Patients with aortic stenosis and mid-wall late gadolinium enhancement (LGE) had a two-fold increase in cardiac troponin I concentrations compared with those without LGE and age- and sex-matched healthy patients.

cTnI concentrations were associated with AVR or cardiovascular deaths, independent of the burden of coronary atherosclerosis (as assessed using coronary calcium scoring) as well as age, sex,

Table 3 Univariate and multivariable linear regression analysis to examine association of variables with plasma cardiac troponin I concentrations

Variables	Univariate		Multivariable—Model 1 (included %LGE)		Multivariable—Model 2 (included ECV)	
	Relative change in troponin I concentration (95% CI)	*P*-value	Relative change in troponin I concentration (95% CI)	*P*-value	Relative change in troponin I concentration (95% CI)	*P*-value
Age, per 10 years	1.32 (1.07–1.44)	0.004	1.49 (1.14–1.80)	0.002	1.36 (1.09–1.72)	0.006
Male sex	1.31 (0.90–1.92)	0.16	0.79 (0.44–1.42)	0.44	0.80 (0.46–1.39)	0.42
Diabetes mellitus	0.92 (0.52–1.61)	0.76				
Hypertension	1.21 (0.83–1.74)	0.33				
CAD	1.20 (0.82–1.75)	0.35	1.01 (0.58–1.73)	0.96	1.17 (0.72–1.91)	0.53
MPG, per 10 mmHg	1.17 (1.02–1.35)	0.02	0.92 (0.79–1.06)	0.27	0.93 (0.73–1.06)	0.28
Mean e′, cm/s	0.86 (0.78–0.95)	0.002	1.08 (0.93–1.27)	0.30	1.02 (0.88–1.19)	0.78
Indexed LVM, per 10 g/m^2	1.23 (1.15–1.32)	<0.001	1.34 (1.15–1.55)	<0.001	1.41 (1.23–1.62)	<0.001
%LGE, %	1.13 (1.08–1.17)	<0.001	1.11 (1.03–1.19)	0.006		
ECV, %	1.15 (1.07–1.23)	<0.001			1.11 (1.00–1.21)	0.05

See *Table 1* for abbreviations.

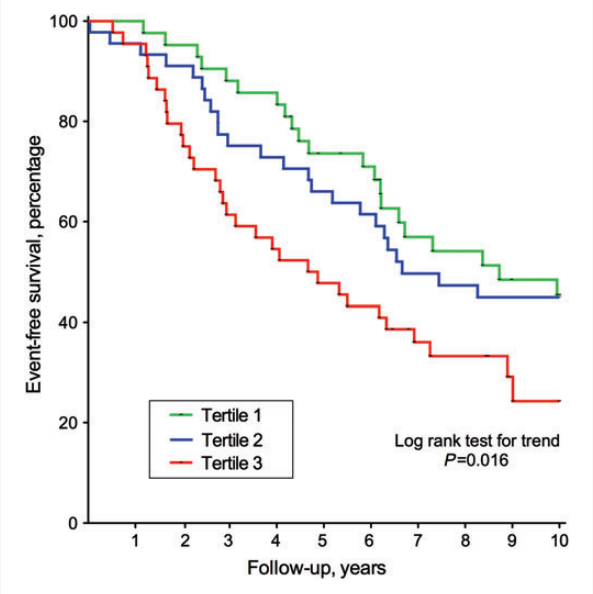

Figure 4 Ten-year event-free survival for composite endpoint of aortic valve replacement or cardiovascular death by tertiles of cardiac troponin I concentrations. Patients in the highest tertile were associated with lower survival rates compared with patients in the other tertiles (log rank test for trend, $P = 0.016$).

Table 4 Hazard ratios predicting time to valve replacement or cardiovascular death for troponin I concentrations in adjusted and unadjusted analyses

Model	Hazard ratio (95% CI)	*P*-value
Model 1	1.65 (1.15–2.38)	0.007
Model 2	1.61 (1.11–2.35)	0.01
Model 3	1.63 (1.11–2.38)	0.01
Model 4	1.77 (1.22–2.55)	0.002
Model 5	2.10 (1.22–3.61)	0.007

Model 1—unadjusted; Model 2—adjusting for age and sex; Model 3—as model 2 additionally adjusting for systolic ejection fraction; Model 4—as model 2 additionally adjusting for mean pressure gradient; Model 5—as model 2 additionally adjusting for coronary and aortic valve calcium score.

systolic ejection fraction, echocardiographic measures of aortic stenosis severity, and the aortic valve calcium score.

A recent study demonstrated an association between high-sensitivity cardiac troponin T concentrations and echocardiographic measures of LV modelling in aortic stenosis.[21] Our data confirm and extend these findings using CMR, which has allowed us to investigate the remodelling response in greater detail and crucially assess the relationship with myocardial fibrosis, thereby providing additional mechanistic data. We therefore believe that the plasma cTnI concentration measured by a high-sensitivity assay has considerable potential as an early biomarker of LV decompensation and as a powerful prognostic tool in patients with aortic stenosis. Moreover, this test is inexpensive and easy to perform making any future transition into routine clinical practice readily achievable. However, considerable overlap was observed between patients with aortic stenosis and our control cohort. This is perhaps unsurprising, given cTnI is released as a consequence of a wide range of myocardial insults. A future strategy where asymptomatic aortic stenosis patients with elevated or increasing plasma troponin concentrations subsequently proceed to CMR for confirmation of myocardial fibrosis and LV

decompensation is therefore attractive. Large-scale prospective studies are now required to investigate the use of these two biomarkers in the management and risk stratification of patients with aortic stenosis and whether the above approach might identify asymptomatic patients who would benefit from early surgery.

In contrast to troponin, BNP did not have prognostic value in our study. BNP is an endogenous cardiac hormone released in response to increasing LV wall stress and most commonly used in the assessment of patients with heart failure. It is therefore only likely to be released late in the transition from hypertrophy to heart failure, making it of limited value in detecting signs of early decompensation in asymptomatic patients. Given that this is the group in whom novel biomarkers of LV decompensation are most likely to be useful, we believe that troponin holds greater clinical promise than BNP.

Limitations

CMR was not available at the inception of the SALTIRE study. Therefore, we needed to recruit another patient population to investigate the mechanism for troponin release in aortic stenosis. However, plasma cTnI concentrations in the Outcome Cohort also displayed a close association with LV mass determined by echocardiography and were unrelated to the burden of coronary atherosclerosis or the severity of valvular stenosis. Similar mechanisms would therefore seem to govern cTnI release across both groups. Another limitation is the lack of more sensitive markers of LV systolic dysfunction in the Mechanism Cohort, for example CMR tagging techniques. However, we elected not to perform these due to concerns about lengthening the scanning protocol in this elderly cohort of patients and compromising the detection of myocardial fibrosis. Finally, data on short-term biological variability (the change in concentration from one occasion to another) are very limited in disease states. However, we do not anticipate significant short-term variability in chronic conditions such as aortic stenosis, although this will require further validation.

Conclusions

In patients with aortic stenosis, plasma cTnI concentrations are a marker of LV decompensation and myocardial fibrosis that are associated with cardiovascular deaths and AVR. High-sensitivity troponin assays hold major promise as a future clinical tool for patients with this condition.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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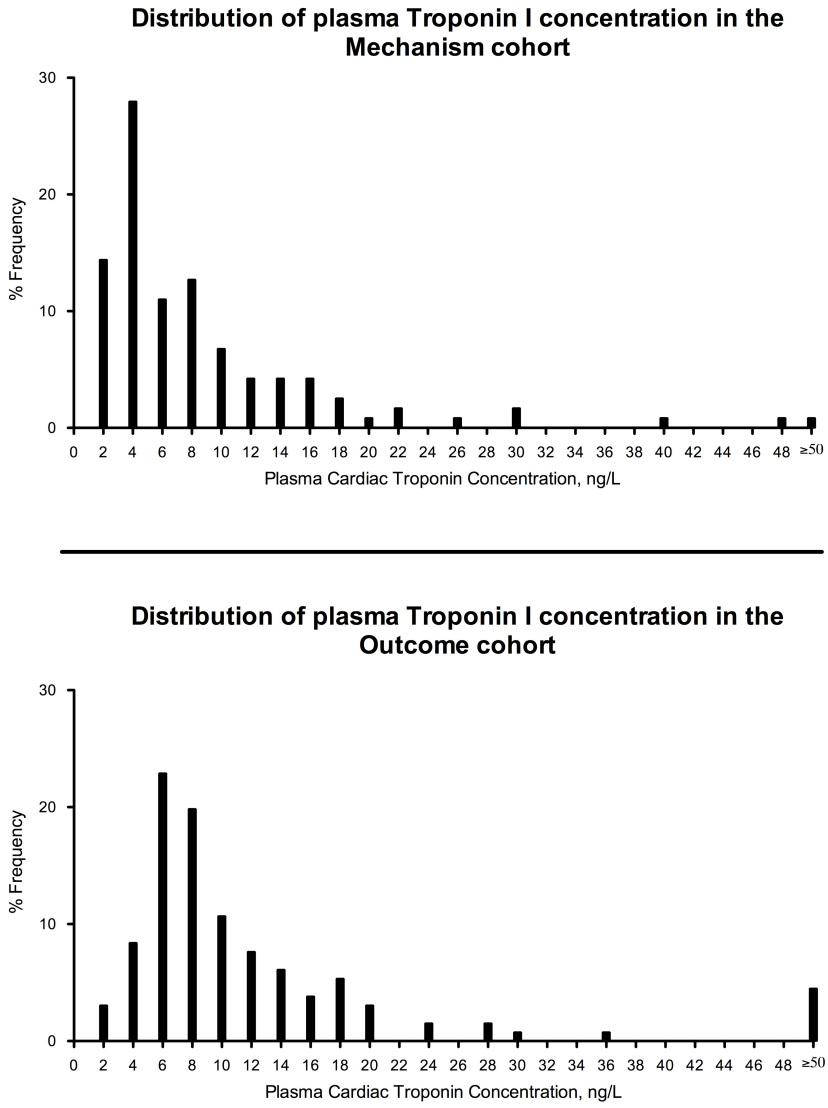
HIGH-SENSITIVITY TROPONIN I CONCENTRATIONS ARE A MARKER OF AN ADVANCED HYPERTROPHIC RESPONSE AND ADVERSE OUTCOMES IN PATIENTS WITH AORTIC STENOSIS

ONLINE SUPPLEMENTAL DATA

1. Distribution of high-sensitivity plasma cardiac troponin I concentrations in the Mechanism and Outcome cohorts of patients with aortic stenosis

Plasma cardiac troponin I concentrations were above the limit of detection in 98% of our patients with aortic stenosis in both cohorts, and exceeded the recommended diagnostic threshold for myocardial infarction (> 26 ng/L) in 8%. The distribution of plasma troponin I concentrations in the two cohorts was similar (Figure S1).

FIGURE S1



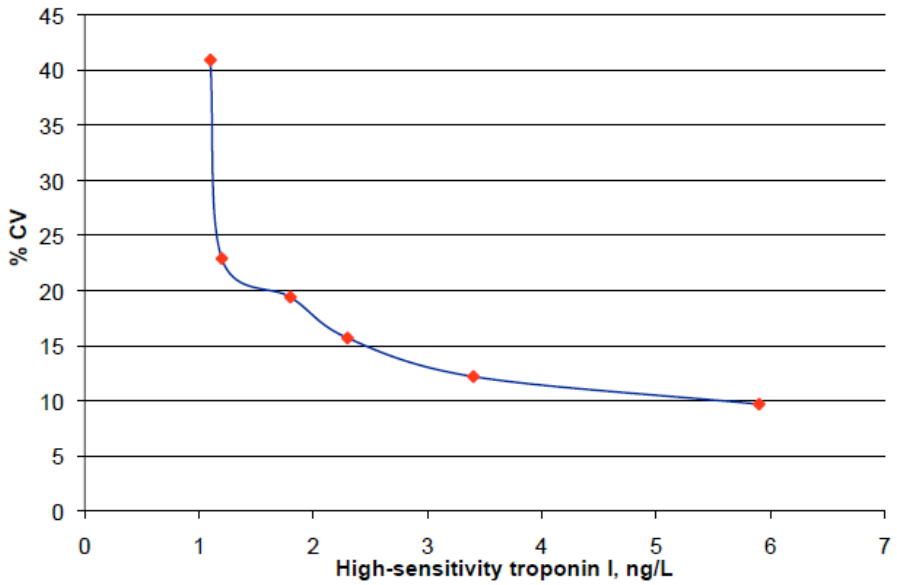
2. Exploratory analysis of 1 year change in cardiac troponin I concentrations and adverse outcomes at 3 and 5 years

Among a subset of patients in whom troponin was measured more than once and who were event-free at one year, we explored the association between both baseline troponin and change in troponin from baseline to year 1 with the odds of valve surgery or death during subsequent follow-up.

Serial plasma cardiac troponin I concentrations were available for 69 patients (52%). Cardiac troponin I (cTnI) concentrations at baseline and one year were strongly correlated (r=0.87, P<0.001). Sixteen (23%) patients had an event within 3 years and 25 (41%) had an event within 5 years of follow-up. Associations in the same direction were evident for both 3-year and 5-year events for both baseline troponin (two-fold increase OR 1.73 (1.11-2.89), P=0.02 and OR 1.39 (0.95-2.16), P=0.11, respectively) and difference in troponin from baseline (2-fold increase OR 3.19 (1.33-8.62), P=0.01 and 1.58 (0.75-3.47), P=0.23, respectively) although the associations met the conventional cut-off for statistical significance for 3-year event rates only.

3. Analytical variability of high-sensitivity cardiac troponin I assay

Precision profiling of the ARCHITECT *STAT* high-sensitivity troponin I assay was performed in 248 samples across 18 healthy controls. The inter-assay coefficient of variation (CV) for duplicate samples is 10% at 6 ng/L and 20% at 1.5 ng/L.



4. Mechanism and prognosis of brain natriuretic peptide in patients with aortic stenosis

In the Mechanism Cohort, brain natriuretic peptide (BNP) concentration was increased in patients with coronary artery disease (Figure S2) and worse symptoms (Figure S3); and associated with age, severity of aortic stenosis, increased left ventricular mass index, diastolic dysfunction and extent of myocardial fibrosis. However, only age was strongly associated with BNP concentration in both models on the multivariable analysis (Table S1). In the Outcome Cohort, N-terminal pro-BNP was not associated with adverse cardiac events with both unadjusted and adjusted analyses (Table S2).

FIGURE S2

In patients with aortic stenosis, brain natriuretic peptide (BNP) concentration was increased in patients with coronary artery disease (CAD)

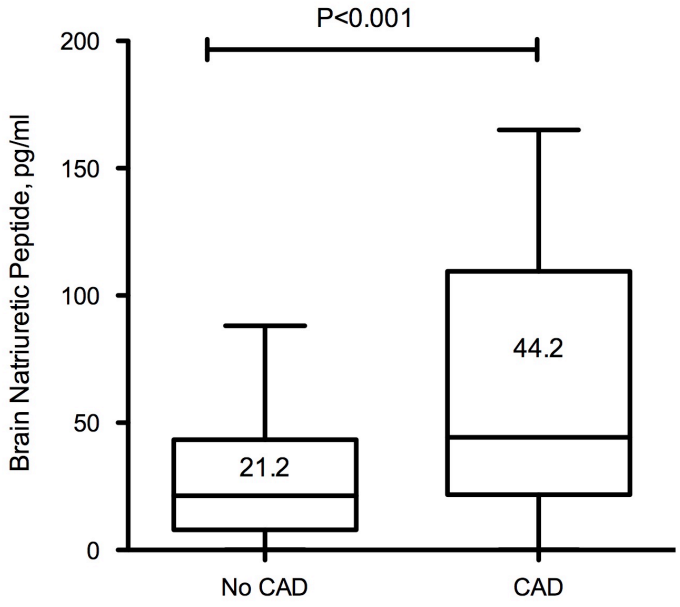


FIGURE S3

Brain natriuretic peptic (BNP) concentrations were increased across New York Heart Association (NYHA) class in patients with aortic stenosis. Of note, all patients in NYHA III had detectable BNP concentrations (range 8.1 to 444 pg/ml).

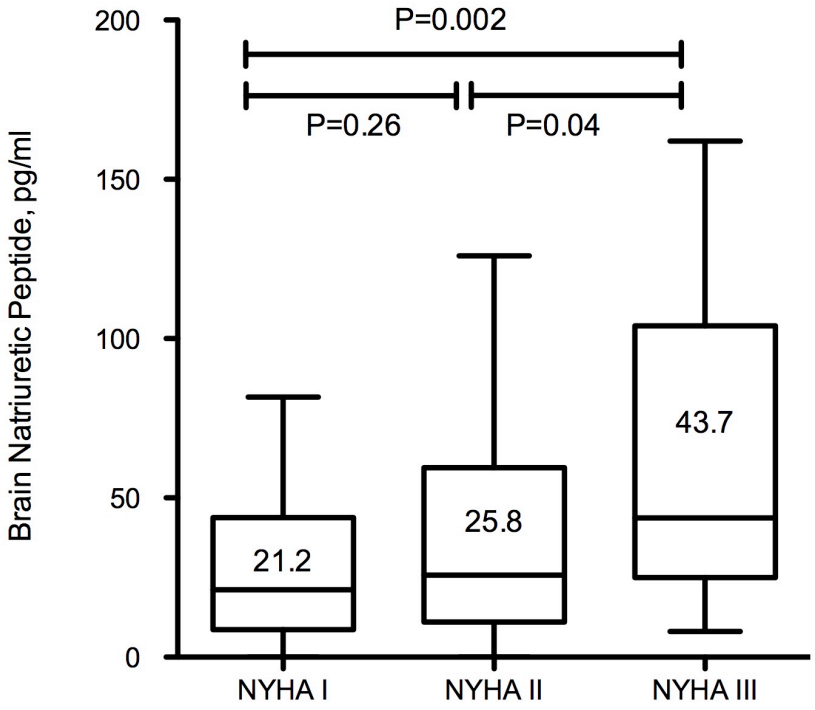


TABLE S1

Univariate and multivariate analyses to examine the determinants of brain natriuretic peptides concentrations in patients with aortic stenosis

Variables	Univariate		Multivariate – Model 1 (Included %LGE)		Multivariate – Model 2 (Included ECV)	
	Relative change in BNP concentration (95% CI)	P value	Relative change in BNP concentration (95% CI)	P value	Relative change in BNP concentration (95% CI)	P value
Age, per 5 years	1.48 (1.35 to 1.62)	<0.001	1.95 (1.82 to 2.36)	<0.001	1.93 (1.58 to 2.34)	<0.001
Male sex	1.12 (0.63 to 1.99)	0.71				
CAD	2.97 (1.75 to 5.10)	<0.001	1.42 (0.91 to 2.24)	0.17	1.86 (1.20 to 2.89)	0.01
Creatinine, per 10 μmol/l	1.17 (1.00 to 1.35)	0.05				
NYHA class	1.77 (1.26 to 2.48)	0.001	1.12 (0.84 to 1.49)	0.43	1.14 (0.85 to 1.54)	0.37
MPG, per 10 mmHg	1.22 (1.06 to 1.42)	0.007	1.08 (0.95 to 1.02)	0.25	1.09 (0.96 to 1.25)	0.16
Mean E/e'	1.08 (1.05 to 1.13)	<0.001	1.02 (0.99 to 1.05)	0.15	1.03 (1.00 to 1.70)	0.07
Ejection fraction, per 5%	0.95 (0.78 to 1.16)	0.60				
Indexed LVM, per 10 g/m^2	1.15 (1.02 to 1.28)	0.02	1.06 (0.95 to 1.02)	0.21	1.11 (1.01 to 1.25)	0.04
%LGE, %	1.12 (1.04 to 1.19)	0.002	1.05 (0.99 to 1.12)	0.12		
ECV, %	1.21 (1.08 to 1.39)	0.001			1.08 (0.97 to 1.18)	0.07

TABLE S2

Hazard ratios for time to valve replacement or cardiovascular death for N-terminal pro-brain natriuretic peptide concentrations in adjusted and unadjusted analyses

Model	Hazard ratio (95%CI)	P value
Model 1[*]	1.15 (0.86 to 1.53)	0.34
Model 2[†]	1.14 (0.80 to 1.60)	0.47
Model 3[‡]	1.13 (0.80 to 1.60)	0.49

[*]Model 1 – unadjusted; [†]Model 2 – adjusting for age and sex; [‡]Model 3 – as Model 2 with additional adjusting for dyspnea (NYHA >1)

Left Ventricular Hypertrophy With Strain and Aortic Stenosis

Anoop S.V. Shah, Calvin W.L. Chin, Vassilis Vassiliou, S. Joanna Cowell, Mhairi Doris, T'ng Choong Kwok, Scott Semple, Vipin Zamvar, Audrey C. White, Graham McKillop, Nicholas A. Boon, Sanjay K. Prasad, Nicholas L. Mills, David E. Newby and Marc R. Dweck

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Left Ventricular Hypertrophy With Strain and Aortic Stenosis

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Background—ECG left ventricular hypertrophy with strain is associated with an adverse prognosis in aortic stenosis. We investigated the mechanisms and outcomes associated with ECG strain.

Methods and Results—One hundred and two patients (age, 70 years [range, 63–75 years]; male, 66%; aortic valve area, 0.9 cm^2 [range, 0.7–1.2 cm^2]) underwent ECG, echocardiography, and cardiovascular magnetic resonance. They made up the mechanism cohort. Myocardial fibrosis was determined with late gadolinium enhancement (replacement fibrosis) and T1 mapping (diffuse fibrosis). The relationship between ECG strain and cardiovascular magnetic resonance was then assessed in an external validation cohort (n=64). The outcome cohort was made up of 140 patients from the Scottish Aortic Stenosis and Lipid Lowering Trial Impact on Regression (SALTIRE) study and was followed up for 10.6 years (1254 patient-years). Compared with those without left ventricular hypertrophy (n=51) and left ventricular hypertrophy without ECG strain (n=30), patients with ECG strain (n=21) had more severe aortic stenosis, increased left ventricular mass index, more myocardial injury (high-sensitivity plasma cardiac troponin I concentration, 4.3 ng/L [interquartile range, 2.5–7.3 ng/L] versus 7.3 ng/L [interquartile range, 3.2–20.8 ng/L] versus 18.6 ng/L [interquartile range, 9.0–45.2 ng/L], respectively; $P<0.001$) and increased diffuse fibrosis (extracellular volume fraction, 27.4±2.2% versus 27.2±2.9% versus 30.9±1.9%, respectively; $P<0.001$). All patients with ECG strain had midwall late gadolinium enhancement (positive and negative predictive values of 100% and 86%, respectively). Indeed, late gadolinium enhancement was independently associated with ECG strain (odds ratio, 1.73; 95% confidence interval, 1.08–2.77; $P=0.02$), a finding confirmed in the validation cohort. In the outcome cohort, ECG strain was an independent predictor of aortic valve replacement or cardiovascular death (hazard ratio, 2.67; 95% confidence interval, 1.35–5.27; $P<0.01$).

Conclusion—ECG strain is a specific marker of midwall myocardial fibrosis and predicts adverse clinical outcomes in aortic stenosis. **(*Circulation*. 2014;130:1607-1616.)**

Key Words: aortic valve stenosis ■ fibrosis ■ hypertrophy, left ventricular ■ troponin I

Aortic stenosis is characterized by progressive valve narrowing of and secondary changes in the myocardium.[1] In response to increased afterload, left ventricular hypertrophy (LVH) can develop to maintain wall stress and cardiac function. Although this process appears to be compensatory in the early stages, preclinical studies have suggested that cardiac performance can be preserved in the absence of hypertrophy.[2,3] Moreover, the LVH response ultimately decompensates with progressive cell death and fibrosis, driving the transition to symptoms, heart failure, and adverse cardiovascular events.[1,4,5] There is therefore considerable interest in identifying early, objective markers of this decompensation that might identify asymptomatic patients who would benefit from early valve replacement.

Clinical Perspective on p 1616

ECG strain is a well-recognized marker of LVH. However, the exact mechanism underlying the characteristic ST- and T-wave abnormalities associated with this pattern is uncertain. In this study, we hypothesized that ECG strain is a marker of

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left ventricular decompensation and investigated this association using cardiovascular magnetic resonance (CMR) to assess the degree of LVH and myocardial fibrosis and high-sensitivity plasma cardiac troponin I (cTnI) as a marker of myocardial injury. Moreover, we aimed to reassess the adverse prognosis previously associated with the ECG strain pattern in patients with aortic stenosis.[6]

Methods

Three cohorts were used for the study. In the mechanism cohort, we determined the pathophysiology underlying the ECG strain pattern using CMR and plasma cTnI concentration in patients recruited from the Edinburgh Heart Center. This was then independently validated in an external validation cohort from the Royal Brompton Hospital, London. Subsequently, in the outcome cohort, we examined the prognostic role of ECG strain in patients with aortic stenosis. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local research ethics committee. Written informed consent was obtained from all participants.

Patient Populations

Mechanism Cohort

Patients with aortic stenosis (mild to severe) were recruited prospectively from the Edinburgh Heart Center. We excluded patients with other significant valvular heart disease (moderate or severe), contraindications to CMR, cardiomyopathies (acquired or inherited), left or right bundle-branch block, concurrent digoxin use, and impaired systolic function on CMR (ejection fraction <95th percentile for age and sex).[7]

Validation Cohort

Between 2011 and 2013, patients with moderate to severe aortic stenosis undergoing CMR were prospectively recruited from the Royal Brompton Hospital, London, with the use of similar exclusion criteria.

Outcome Cohort

Patients were initially recruited into the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE) study between March 2001 and April 2002, which comprised 155 patients with asymptomatic aortic stenosis who had been randomly assigned to either atorvastatin 80 mg or placebo once daily. Patients were excluded if already on a statin or if aortic valve replacement (AVR) was planned (because of either symptoms or impaired systolic function).[8] For the purposes of this analysis, patients on digoxin or with uninterpretable ECGs or bundle-branch block patterns were excluded.

Electrocardiography

A standard 12-lead ECG was obtained for all participants, and interpretation of the ECG was performed independently by 2 observers who were blinded to the clinical data and imaging findings. LVH on ECG was diagnosed on the basis of the Romhilt-Estes point system (≥5 points),[9] and ECG strain was defined as ≥1-mm concave downsloping ST-segment depression with asymmetrical T-wave inversion in the lateral leads (Figure 1A).[10]

Echocardiography

Transthoracic echocardiography was performed in all participants in the mechanism and outcome cohorts. Maximum aortic valve jet velocity and mean pressure gradient were measured by velocity–time integral spectral tracing and the aortic valve area derived with the continuity equation. Multiple acoustic windows with the S51 and

Figure 1. ECGs and myocardial biopsies in 2 patients with severe aortic stenosis. The ECG for patient A (**A**) demonstrated left ventricular hypertrophy and associated repolarization abnormalities (ST-segment depression and asymmetrical T-wave inversion in the lateral leads) characteristic of the ECG strain pattern, whereas the ECG for patient B (**B**) demonstrated left ventricular hypertrophy without the strain pattern. Compared with patient B, patient A had increased left ventricular mass index (169 vs 81 g/m^2), increased plasma cardiac troponin I concentrations (8.4 vs 2.5 ng/L), and evidence of more extensive myocardial fibrosis on both cardiovascular magnetic resonance and histology (picrosirius red staining).

D2cwc probes (Philips Medical Systems, Best, the Netherlands) were assessed. The severity of aortic stenosis was classified according to the European Association of Echocardiography/American Society of Echocardiography guidelines.[11] Transmitral early (E) and late (A) diastolic velocities and deceleration time of early filling velocity were measured at the tips of the mitral valve leaflets with pulsed-wave Doppler. Early (e′) diastolic velocities of the medial and lateral mitral annulus were measured with pulsed-wave tissue Doppler imaging. Diastolic function was determined from the E/A ratio, deceleration time, mean of medial and lateral e′, and E/e′. Midwall fractional shortening was estimated as an assessment of intrinsic myocardial contractility in the context of LVH.[12]

Cardiovascular Magnetic Resonance

CMR in the mechanism cohort was performed at 3 T (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany). In the validation cohort, CMR was performed at 1.5 T, as previously described.[13] For the assessment of left ventricular function and mass, short-axis cine images from the mitral valve annulus to the apex were obtained by use of a balanced steady-state free-precession sequence (8-mm parallel slices with 2-mm spacing). The quantification of left ventricular function, volumes, and mass was assessed with dedicated software (Siemens AG Healthcare Sector, Erlangen, Germany) and indexed to body surface area. LVH on CMR was defined as a left ventricular mass index (LVMi) >95th percentile using age- and sex-specific normal ranges.[7] Left ventricular longitudinal shortening was determined by measuring the difference in mitral annular displacement between end systole and end diastole. The mean value of the lateral and septal insertion sites (4-chamber view) and the anterior and inferior sites (2-chamber view) was used.

The assessment of focal replacement myocardial fibrosis was performed with late gadolinium enhancement (LGE) imaging, 15 minutes after administration of 0.1 mmol/kg gadobutrol (Gadovist/Gadavist, Bayer Pharma AG, Berlin, Germany). Two approaches were used: an inversion recovery fast gradient-echo sequence and a phase-sensitive inversion recovery sequence, performed in 2 phase-encoding directions to differentiate true late enhancement from artifact. The inversion time was optimized to achieve satisfactory nulling of the myocardium. Midwall LGE was determined visually by 2 independent operators who were blinded to the ECG findings. The amount of LGE was quantified with QMASS software (Medis Medical Imaging Systems, Leiden, the Netherlands) using a signal intensity threshold greater than twice the standard deviation above the mean value in a normal region of myocardium sampled on the same short-axis image. Areas thought to represent inversion artifact or contamination by blood pool or epicardial fat were manually excluded.

Table 1. Baseline Characteristics of Patients in the Mechanism Cohort

		Patients With Aortic Stenosis			
	All Patients (n=102)	No LVH (n=51)	LVH Without Strain (n=30)	ECG Strain (n=21)	*P* Value
Clinical characteristics					
Age, y	70 (63–75)	70 (65–75)	70 (65–73)	69 (61–75)	0.83
Male sex, n (%)	67 (66)	30 (59)	23 (77)	16 (76)	0.09
Diabetes mellitus, n (%)	8 (8)	5 (10)	2 (7)	2 (10)	0.88
Hypertension, n %	61 (60)	29 (58)	21 (70)	11 (52)	0.99
Coronary artery disease, n (%)	28 (27)	11 (22)	9 (30)	8 (38)	0.14
Systolic blood pressure, mm Hg	146±21	146±22	146±20	147±20	0.98
Bicuspid aortic valve, n (%)	33 (32)	13 (25)	12 (40)	8 (38)	0.21
NYHA class III and IV, n (%)	22 (22)	4 (8)	6 (20)	12 (57)	<0.001
ECG					
Aortic valve area, cm^2	0.9 (0.7–1.2)	1.0 (0.7–1.3)	0.9 (0.7–1.1)	0.7 (0.6–0.9)	0.02
Aortic jet velocity, m/s	3.8±1.0	3.2±0.7	3.8±0.8	4.8±1.1	<0.001*†‡
MPG, mm Hg	30 (20–39)	23 (14–32)	31 (22–41)	45 (37–64)	<0.001
Dimensionless index	0.29±0.10	0.31±0.10	0.28±0.09	0.25±0.09	0.03‡
Midwall fractional shortening, mm	10.9±1.7	11.2±1.9	10.9±1.5	9.9±1.2	0.01‡
Mitral E/A ratio	1.0±0.4	0.9±0.4	1.0±0.4	0.9±0.4	0.51
Deceleration time, ms	210±56	197±51	214±57	235±57	0.02
Mean e′, cm/s	6.2±1.9	6.7±2.0	6.3±1.7	4.9±1.5	<0.01‡
E/e′ ratio	12.5 (10.0–16.7)	11.7 (9.4–14.9)	12.3 (9.3–15.4)	17.0 (13.0–23.0)	<0.001†‡
Cardiac MRI					
Indexed LV mass, g/m^2	91±24	75±14	99±18	118±22	<0.001*†‡
LV mass/EDV ratio, g/mL	1.27±0.26	1.14±0.22	1.33±0.21	1.51±0.22	<0.001*†‡
Indexed EDV, mL/m^2	69 (62–78)	67 (60–71)	77 (69–88)	73 (65–86)	<0.001
Indexed ESV, mL/m^2	22 (18–27)	22 (17–25)	25 (21–29)	23 (19–29)	0.03
Indexed stroke volume, mL	49±10	45±8	51±10	54±12	<0.01*‡
Ejection fraction, %	68±6	68±5	67±6	68±8	0.90
Longitudinal shortening, mm	12.4±3.1	13.1±2.7	12.9±3.1	9.9±2.7	<0.001†‡
Patients with midwall LGE, n (%)	32 (31)	4 (8)	7 (23)	21 (100)	<0.001
Amount of LGE, %	0 (0–5.5)	3.9 (1.8–7.0)	5.8 (5.0–7.6)	9.5 (7.5–14.2)	<0.01
ECV, %	28.1±2.8	27.4±2.2	27.2±2.9	30.9±1.9	<0.001†‡
Plasma high-sensitivity cTnI concentration, ng/L	6.7 (3.6–13.3)	4.3 (2.5–7.3)	7.3 (3.2–20.8)	18.6 (9.0–45.2)	<0.001

cTnI indicates cardiac troponin I; ECV, extracellular volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; LGE, late gadolinium enhancement; LV, left ventricular; LVH, left ventricular hypertrophy; MPG, mean pressure gradient; MRI, magnetic resonance imaging; and NYHA, New York Heart Association.

ANOVA post hoc Bonferroni adjustment: *P<0.05 between no LVH and LVH without strain, †P<0.05 between LVH without strain and LVH with strain, and ‡P<0.05 between no LVH and LVH with strain.

Myocardial extracellular volume fraction (ECV) has been demonstrated to act as a measure of diffuse myocardial fibrosis in a variety of cardiac conditions, including aortic stenosis.[14–16] Recently, we have described a standardized approach to analyze myocardial ECV in patients with aortic stenosis, demonstrating excellent intraobserver, interobserver, and scan-rescan reproducibility of ±3%.[17] In brief, myocardial T1 mapping was performed in the mechanism cohort using the modified look-locker inversion recovery sequence: flip angle, 35°; minimum TI, 100 milliseconds; TI increment, 80 milliseconds; and time delay, 150 milliseconds with a heartbeat acquisition scheme of 3-3-5.[18] Regions of interest were drawn around the myocardium on the short-axis, precontrast, motion-corrected myocardial T1 maps and copied onto corresponding 20-minute postcontrast maps, with minor adjustments made to avoid partial volume effects and artifact (OsiriX version 4.1.1, Geneva, Switzerland). ECV was calculated according to the following formula: $ECV=(\Delta R1_{myocardium}/\Delta R1_{blood\text{-}pool})\times(1-\text{hematocrit})$, where $\Delta R1=(1/\text{postcontrast T1}-1/\text{precontrast T1})$. Hematocrit was sampled at the time of CMR.

High-Sensitivity Plasma cTnI Assay

Plasma cTnI concentrations were determined in patients in the mechanism cohort as a marker of myocyte injury with the ARCHITECT$_{STAT}$ high-sensitive troponin I assay (Abbott Laboratories, Abbott Park, IL). Previous data have shown that high-sensitivity plasma cTnI concentrations correspond to the magnitude of the hypertrophic response and extent of myocardial fibrosis in patients with aortic stenosis.[19,20] The lower limit of detection for this assay is 1.2 ng/L, and the 99th percentile from a healthy reference population of 26 ng/L, with a 10% interassay coefficient of variation at 4.7 ng/L.[21] Concentrations lower than the detection limit were assigned a value of 1.2 ng/L.

Calcium Scoring in the Outcome Cohort

ECG-gated noncontrast computed tomography scans of the coronary arteries and aortic valve were performed in all patients in the outcome cohort with a double-helix scanner (Twin II Flash, Philips Medical Systems). Coronary artery and aortic valve calcium scores were determined by a single operator using the Picker Cardiac Scoring software.[8]

Long-Term Follow-Up in the Outcome Cohort

Clinical outcomes were obtained in the outcome cohort and adjudicated by 2 independent investigators blinded to the clinical and ECG data. In-hospital and community deaths were captured from the General Register of Scotland. Cardiovascular death was defined as death resulting from myocardial infarction, sudden cardiac death, heart failure, or stroke; death related to cardiovascular procedures; and death resulting from other cardiovascular causes. Each death was classified by the 2 independent investigators, and any discrepancy was resolved by consensus. Furthermore, all events, including surgical AVR (no patients had transcatheter aortic valve implantation during follow-up), were confirmed by independent review of each patient's healthcare record. All patients in the outcome cohort were managed in our tertiary cardiac center and reviewed at a multidisciplinary meeting before undergoing AVR. Only patients with established indications as per contemporary guidelines were referred for AVR.[22,23]

Statistical Analysis

Continuous variables were presented as mean±SD or median (interquartile range) as appropriate. The distribution of all continuous variables was assessed for normality with the Shapiro-Wilk test. Comparison of continuous variables was performed with the Student *t* test or 1-way ANOVA with post hoc Bonferroni adjustment when appropriate. The assumption of homogeneity of variances was tested with the Levene test. For nonparametric data, the Mann-Whitney *U* or Kruskal-Wallis test was used. Categorical variables were expressed as percentages and compared by use of the χ^2 test for trend. All statistical analyses were performed with

Table 2. Baseline Characteristics of Patients in the External Validation Cohort

		Patients With Aortic Stenosis			
	All Patients (n=64)	No LVH (n=48)	LVH Without Strain (n=5)	ECG Strain (n=11)	*P* Value
Clinical characteristics					
Age, y	76 (69–84)	78 (68–83)	80 (63–89)	80 (73–87)	0.34
Sex, males, n (%)	44 (69)	32 (67)	4 (80)	8 (73)	0.26
Diabetes mellitus, n (%)	16 (25)	13(27)	0	3 (27)	0.78
Hypertension, n %	33 (52)	26(54)	1 (20)	6 (55)	0.76
Coronary artery disease, n (%)	26 (41)	21(44)	1 (20)	4 (36)	0.51
Systolic blood pressure, mm Hg	133 (119–142)	134 (121–142)	132 (124–158)	123 (110–140)	0.49
Bicuspid aortic valve, n (%)	14 (22)	11 (23)	0	3 (27)	0.97
NYHA class III and IV, n (%)	14 (22)	11 (23)	0	3 (27)	0.97
Race, n (%)					
White	59 (92)	44 (92)	5 (100)	10 (91)	0.95
South Asian	5 (8)	4 (8)	0	1 (9)	
Cardiac MRI					
Planimetered aortic valve area, cm^2	0.9 (0.7–1.0)	0.8 (0.7–1.0)	1.0 (0.7–1.3)	0.7 (0.7–0.9)	0.12
Indexed LV mass, g/m^2	88 (74–113)	85 (72–107)	82 (74–113)	121 (102–133)	0.02
LV mass/EDV ratio, g/mL	1.17 (0.86–1.38)	1.18 (0.91–1.35)	1.05 (0.88–1.42)	1.13 (0.79–1.48)	0.92
Indexed EDV, mL/m^2	76 (67–104)	74 (64–95)	86 (67–97)	120 (75–153)	0.01
Indexed ESV, mL/m^2	28 (17–48)	27 (17–42)	29 (17–36)	65 (38–102)	<0.01
Indexed stroke volume, mL	48 (38–55)	47 (40–54)	55 (47–65)	49 (36–52)	0.19
Ejection fraction, %	64 (46,72)	65 (51,73)	68 (63–76)	44 (32–48)	<0.01
Patients with midwall LGE, n (%)	25 (39)	12 (25)	3 (60)	10 (91)*	0.02

EDV indicates end-diastolic volume; ESV, end-systolic volume; LGE, late gadolinium enhancement; LV, left ventricular; LVH, left ventricular hypertrophy; MRI, magnetic resonance imaging; and NYHA, New York Heart Association.

*The remaining patient had a large infarct.

GraphPad Prism (GraphPad Software Inc, San Diego, CA), R version 2.15.2 (Vienna, Austria), and SPSS version 20.0.0 (IBM Corp, Armonk, NY). A 2-sided value of $P<0.05$ was considered statistically significant.

Mechanism Cohort

In the mechanism cohort, the association between ECG strain and left ventricular mass and aortic stenosis severity was assessed with multivariable linear regression analysis to adjust for potential confounders. Furthermore, we assessed determinants associated with ECG strain using univariate and multivariable logistic regression.

Outcome Cohort

In the outcome cohort, time-to-event curves in patients with and without ECG strain were estimated with the Kaplan–Meier method and compared by use of the log-rank test. To accommodate for competing risks, the association between time to AVR or cardiovascular death and ECG strain was modeled as a composite outcome in Cox proportional hazards models. The assumption for proportional hazards was assessed using the log (−log[survival]) versus log(survival time) plots and by examining the Schoenfeld residuals.

Results

One hundred and two patients with aortic stenosis (age, 70 years [interquartile range, 63–75 years]; male, 66%; aortic valve area, 0.9 cm^2 [interquartile range, 0.7–1.2 cm^2]) were recruited into the mechanism cohort, and an additional 64 patients were recruited into the validation cohort (age, 76 years [interquartile range, 69–84 years]; male, 69%; aortic valve area, 0.9 cm^2 [interquartile range, 0.7–1.0 cm^2]; Tables 1 and 2). After the exclusion of patients with uninterpretable ECGs or bundle-branch block and those receiving digoxin therapy (n=15), 140 patients from the SALTIRE study were analyzed as part of the outcome cohort (age, 69 years [interquartile range, 62–75] years; male, 70%; aortic valve area, 1.0 cm^2 [interquartile range, 0.7–1.3 cm^2]; Table 3). All patients in the mechanism and outcome cohorts were white. In the validation cohort, 92% were white and the remainder were South Asian. There were no observed racial differences with respect to the presence of LVH or strain on the ECG ($P=0.95$; Table 2).

Mechanisms Underlying ECG Strain

Fifty-one patients in the mechanism cohort fulfilled the ECG criteria for LVH, demonstrating high predictive values for the presence of CMR-defined LVH (positive predictive value, 96%; negative predictive value, 89%). Of these, 21 patients had the strain pattern on their ECGs. These patients with ECG strain had the highest LVMi and most severe aortic stenosis compared with the other patient groups (those without LVH on their ECG and those with LVH but no ECG strain; Table 1), even after adjustment for age, sex, and systolic blood pressure ($P<0.001$ for both). Moreover, compared with other groups, these patients had increased end-diastolic volumes ($P<0.01$), worse diastolic function ($P<0.001$), and more severe symptoms ($P<0.001$; Table 1). Despite similar left ventricular ejection fractions, patients with LVH and ECG strain also had the worst longitudinal function (Figure 2) and midwall fractional shortening (Table 1).

Interestingly, all patients with LVH and ECG strain had focal midwall fibrosis (positive and negative predictive value, 100% and 86%, respectively; Figure 3B), strongly supporting ECG strain as a specific marker of replacement myocardial fibrosis. Moreover, these patients had more extensive diffuse myocardial fibrosis (ECV, 30.9±1.9% versus 27.2±2.9% in patients with LVH and no ECG strain versus 27.4±2.2% in patients without LVH; $P<0.001$; Figure 3A) and myocardial injury as assessed by high-sensitivity plasma cTnI. Indeed, plasma cTnI concentrations were >4-fold higher in patients with strain than in the other patient groups (18.6 ng/L [interquartile range,

Table 3. Characteristics of Patients in the Outcome Cohort

	All Patients (n=140)	Patients With Aortic Stenosis		
		No ECG Strain (n=120)	ECG Strain (n=20)	*P* Value
Clinical characteristics				
Age, y	69 (62–75)	69 (61–75)	75 (66–77)	0.05
Male sex, n (%)	98 (70)	82 (68)	16 (80)	0.43
Diabetes mellitus, n (%)	4 (3)	4 (3)	0	0.59
Hypertension, n (%)	71 (50)	58 (48)	13 (65)	0.23
Coronary artery disease, n (%)	24 (17)	20 (17)	4 (20)	0.75
Systolic blood pressure, mm Hg	144±19	144±20	142±16	0.65
ECG				
Aortic valve area, cm^2	1.0 (0.7–1.3)	1.0 (0.7–1.3)	0.6 (0.4–0.8)	0.03
Peak aortic jet velocity, m/s	3.4 (2.8–4.0)	3.2 (2.8–3.9)	3.9 (3.5–4.4)	<0.01
MPG, mm Hg	24 (18–35)	22 (17–33)	34 (26–44)	<0.001
Ejection fraction, %	69±10	70±11	69±9	0.70
LVMi, g/m^2	173 (142–205)	164 (131–200)	203 (177–223)	<0.01
Computed tomography				
Coronary calcium score, log AU	1.6±1.2	1.6±1.3	1.7±1.2	0.69
Aortic valve calcium score, log AU	3.6±0.6	3.6±0.6	4.0±0.4	0.01
Plasma high-sensitivity cTnI concentration, ng/L	7.5 (5.7–13.4)	6.9 (5.3–11.4)	17.3 (10.5–29.6)	<0.001

AU indicates arbitrary units; cTnI, cardiac troponin I concentration; LVMi, left ventricular mass index; and MPG, mean pressure gradient.

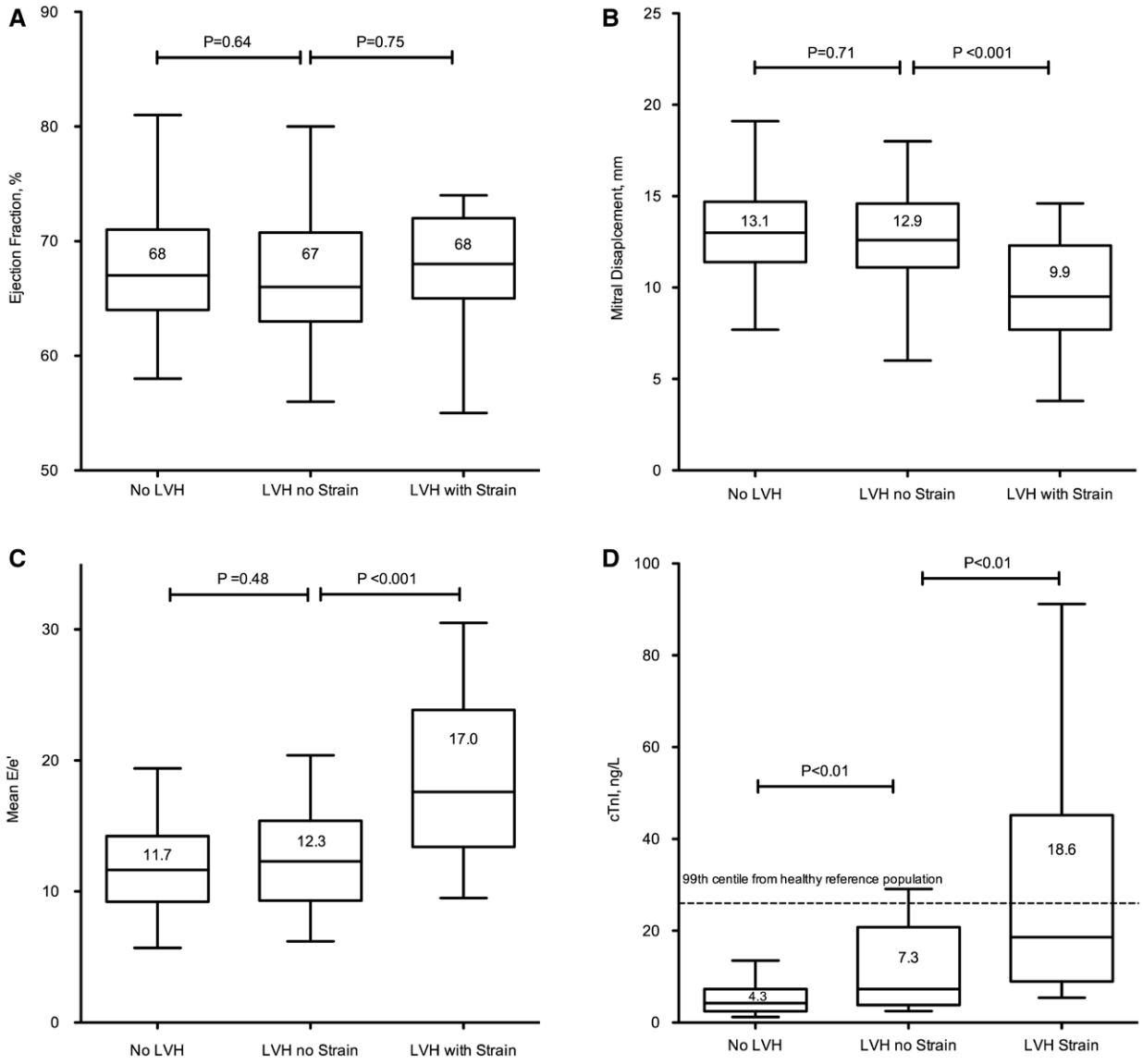

Figure 2. Despite similar normal-range ejection fractions (**A**), patients with left ventricular hypertrophy (LVH) and ECG strain had the most impaired longitudinal shortening (**B**) and diastolic function (**C**). High-sensitivity plasma cardiac troponin I concentrations were 4-fold higher in patients with ECG strain compared with patients without LVH on ECG (**D**). Results are presented in box-and-whiskers plot (Tukey method).

9.0–45.2 ng/L] versus 7.3 ng/L [interquartile range, 3.2–20.8 ng/L] in patients with LVH and no ECG strain versus 4.3 ng/L [interquartile range, 2.5–7.3 ng/L] in patients without LVH; $P<0.001$; Figure 2D). Three patients with ECG strain had both an infarct and midwall pattern of fibrosis on LGE, and our findings remained unchanged even after their exclusion.

On univariate analysis, ECG strain was associated with an increased LVMi, more severe aortic stenosis, increased replacement and diffuse myocardial fibrosis, and diastolic dysfunction (all $P<0.01$; Table 4) but was not associated with the presence of coronary artery disease (odds ratio, 1.88; 95% confidence interval, 0.68–5.18; $P=0.22$). However, only increased myocardial fibrosis (either amount of LGE or ECV) and the severity of aortic stenosis maintained an independent association on multivariate analysis, with increased LVMi, increased myocardial injury, and diastolic dysfunction all dropping out of the model (models 3 and 4 in Table 4).

Myocardial histology was available in 2 patients who underwent AVR and biopsy, supporting increased myocardial fibrosis in patients with LVH and ECG strain (Figure 1). However, not all patients with myocardial LGE had a strain pattern on the ECG. Indeed, of the 32 patients with myocardial LGE, 11 patients (34%) did not have any evidence of ECG repolarization abnormalities. Interestingly, these patients had ≈40% less replacement fibrosis on LGE compared with patients who had ECG strain (5.6% [interquartile range, 4.3%–7.5%] versus 9.5% [interquartile range, 7.5%–14.2%]; $P=0.002$), with no differences in the distribution of midwall LGE between these groups ($P=0.78$; see Distribution of Midwall Fibrosis in the online-only Data Supplement).

Validation Cohort

In the external validation cohort, similar findings were demonstrated (Table 2). There were 11 patients with ECG strain, of whom 10 had isolated midwall fibrosis and 1 had extensive

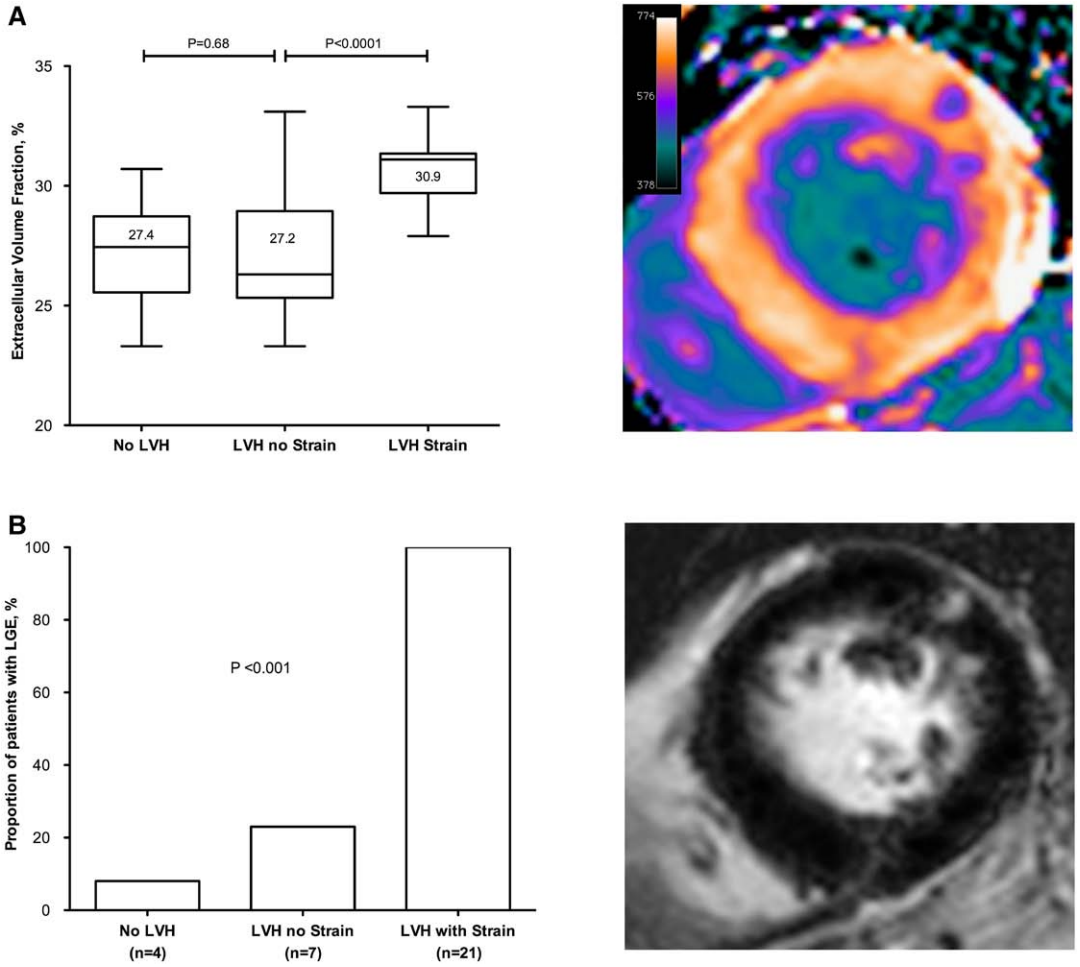

Figure 3. Patients with the strain pattern on the ECG had increased extracellular volume fractions, suggestive of increased diffuse myocardial fibrosis (**A**). Furthermore, all patients with ECG strain had a midwall pattern of late gadolinium enhancement (**B**). Of note, about a third of patients with midwall late gadolinium enhancement did not have ECG strain. The corresponding myocardial T1 map (**A**) and late gadolinium enhancement image (**B**) of a patient with ECG strain demonstrated evidence of focal myocardial fibrosis in the midcavity lateral wall. The extracellular volume fraction of the midcavity slice in this patient was 30.2%. LVH indicates left ventricular hypertrophy.

fibrosis from a large myocardial infarct to explain the ECG changes. Conversely, 15 patients had midwall fibrosis but no ECG strain. In this cohort of patients with moderate to severe aortic stenosis, the positive and negative predictive values of LVH with ECG strain for midwall fibrosis were 91% and 72%, respectively. Patients with ECG strain were again observed to have an advanced hypertrophic response associated with increased LVMi and reduced myocardial performance (Table 2).

Prognostic Value of ECG Strain

In the outcome cohort, 20 patients (14%) had LVH with strain on ECG. Consistent with the mechanism cohort, patients with ECG strain had more severe aortic stenosis, increased LVMi, and elevated plasma cTnI concentrations compared with those without strain (Table 3). Of note, these elevated cTnI concentrations in patients with ECG strain were similar to those observed in the mechanism cohort (P=0.85). Over 10.6 years of follow-up (1254 patient-years), 63 patients had an AVR and 22 patients died of a cardiovascular cause of a total of 36 deaths. ECG strain was associated with a lower 10-year event-free survival rate for AVR or cardiovascular death (log-rank test, P<0.0001; Figure 4). Indeed, this association persisted even after adjustment for traditional markers of an adverse outcome, including systolic ejection fraction, severity of aortic stenosis, LVMi, and aortic valve calcium score (hazard ratio, 2.67; 95% confidence interval, 1.35–5.27; P<0.01; see Univariate and Adjusted Cox Models Predicting Time to Adverse Events in the online-only Data Supplement).

Discussion

This is the first CMR study to investigate the mechanisms underlying the ECG strain pattern in patients with aortic stenosis, demonstrating that it is a highly specific marker of midwall myocardial fibrosis. Moreover, ECG strain was associated with increased myocardial injury and impaired left ventricular performance and was an independent predictor of cardiovascular death or AVR. Our data therefore indicate that ECG strain is a powerful biomarker of left ventricular decompensation in aortic stenosis, with the ability to identify an at-risk population who may benefit from earlier valve replacement.

Currently, AVR is recommended in patients with severe aortic stenosis who have symptoms or evidence of left ventricular decompensation with an ejection fraction <50%.[23] However, symptoms are often subjective, particularly in the elderly, and

Table 4. Univariate and Multivariate Logistic Regression Analyses to Assess Determinants of ECG Strain

	Univariate Analysis		Multivariate Analysis (Model 1)		Multivariate Analysis (Model 2)		Multivariate Analysis (Model 3)	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age, per 10 y	0.91 (0.61–1.36)	0.56	0.88 (0.38–2.03)	0.76	…	…	…	…
Male sex	1.69 (0.56 -5.10)	0.35	0.54 (0.07–3.93)	0.54	…	…	…	…
Coronary artery disease	1.88 (0.68–5.18)	0.22	…	…	…	…	…	…
MPG, per 10 mm Hg	1.80 (1.31–2.48)	<0.001	1.88 (1.02–1.13)	<0.01	1.93 (1.04–3.60)	0.03	2.10 (1.22–3.60)	0.01
LVMi, per 10 g/m^2	2.10 (1.49–2.95)	<0.001	1.95 (1.14–3.35)	<0.01	1.30 (0.63–2.66)	0.47	1.77 (0.97–3.22)	0.06
Amount of LGE, %	1.75 (1.35–2.27)	<0.001	…	…	1.73 (1.08–2.77)	0.02	…	…
ECV, %	1.86 (1.38–2.47)	<0.001	…	…	…	…	1.55 (1.04–2.31)	0.03
High-sensitivity cTnI*	3.14 (1.73–5.71)	<0.001	3.30 (1.24–8.80)	0.02	3.18 (0.62–16.26)	0.16	2.43 (0.83–7.10)	0.11
Mean e′	0.51 (0.34–0.75)	<0.01	…	…	1.71 (0.38–7.54)	0.71	0.95 (0.46–1.94)	0.88

CI indicates confidence interval; cTnI, cardiac troponin I; ECV, extracellular volume fraction; LGE, late gadolinium enhancement; LVMi, left ventricular mass index; MPG, mean pressure gradient; and OR, odds ratio.

*Log-transformed.

a reduced ejection fraction is frequently a late manifestation and is not necessarily reversible. There is therefore interest in exploring alternative, earlier, and more objective markers of ventricular decompensation in aortic stenosis.[5]

Previous echocardiographic studies have demonstrated that ECG strain is associated with an advanced hypertrophic response,[24] and it has been hypothesized that the characteristic repolarization abnormalities relate to coronary perfusion abnormalities, even in the absence of coronary artery disease.[25–28] Our study adds to these data, demonstrating a close association between ECG strain and myocardial injury and fibrosis. Indeed, across 2 independent cohorts, midwall myocardial fibrosis was present in 31 of the 32 patients with strain on their ECGs, and the remaining subject had an extensive infarct to explain the ECG changes. Moreover, patients with strain had evidence of higher plasma cTnI concentrations and worse myocardial function. It has been established that myocardial ischemia, cell death, and fibrosis are all key features that characterize the transition from hypertrophy to heart failure in aortic stenosis. Our study would therefore support ECG strain as a useful marker of left ventricular decompensation in patients with this condition.

In our outcome cohort, we have demonstrated that ECG strain acts as a strong independent predictor of AVR or cardiovascular death, over and above established prognostic markers such as systolic ejection fraction, severity of aortic stenosis, LVMi, and aortic valve calcium score. Indeed, patients with ECG strain had a >2-fold increase risk in adverse events compared with patients without ECG strain. This is in agreement with previous studies that have demonstrated an adverse prognosis associated with ECG strain.[6,29,30] However, our study provides much longer periods of follow-up than have been described previously.

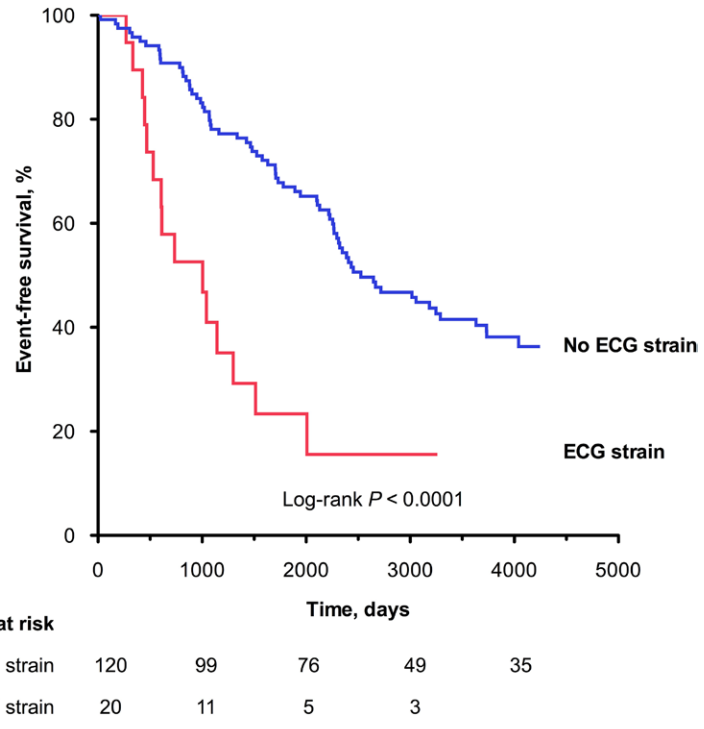

Figure 4. Kaplan–Meier estimates of time to event by status of ECG strain in the outcome cohort. Patients with ECG strain had significantly lower event-free survival compared with patients without ECG strain. LVH indicates left ventricular hypertrophy.

There are clear potential advantages of using ECG strain as a marker of left ventricular decompensation in aortic stenosis. A 12-lead ECG is readily available, cheap, and rapidly interpretable. However, although ECG strain is an extremely specific marker for myocardial fibrosis, it is less sensitive. Indeed, in our mechanism cohort, >30% of patients with replacement myocardial fibrosis did not have strain on the ECG. Importantly, these patients had 40% less replacement fibrosis compared with those with strain, suggesting that strain is a relatively late manifestation and that CMR offers even more sensitive detection of myocardial fibrosis and left ventricular decompensation.

Our data suggest that patients with ECG strain who are asymptomatic would derive long-term benefit from early AVR as a result of the prevention of progressive myocardial fibrosis and injury that would otherwise develop while the patient waited for the onset of symptoms. The stage is now set for randomized, controlled studies to investigate this strategy, examining the clinical utility of the ECG strain pattern in guiding early AVR alongside other novel and more sensitive markers of left ventricular decompensation, including high-sensitivity cTnI concentrations[20] and midwall LGE.[13]

Limitations

In this study, separate cohorts were used to investigate the mechanism and prognosis of patients with ECG strain because CMR was not available in the original SALTIRE study. We therefore cannot directly confirm that ECG strain was similarly related to myocardial fibrosis in the outcome study. However, ECG strain in this population demonstrated the same associations with increased LVMi, aortic stenosis severity, and plasma cTnI concentrations, as observed in the mechanism cohort. Moreover, in our validation cohort, the same clear association between ECG strain and midwall LGE was also observed. We are therefore confident that ECG strain acts as a specific marker of midwall myocardial fibrosis and left ventricular decompensation in the predominantly white patients investigated in this study, although further studies are required for confirmation in different ethnic groups.

Conclusions

In patients with aortic stenosis, ECG strain is a specific marker of midwall myocardial fibrosis and an independent predictor of cardiovascular death or AVR. Future research should now examine whether the ECG strain should be used as a marker of left ventricular decompensation to guide early AVR in asymptomatic patients.

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Disclosures

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CLINICAL PERSPECTIVE

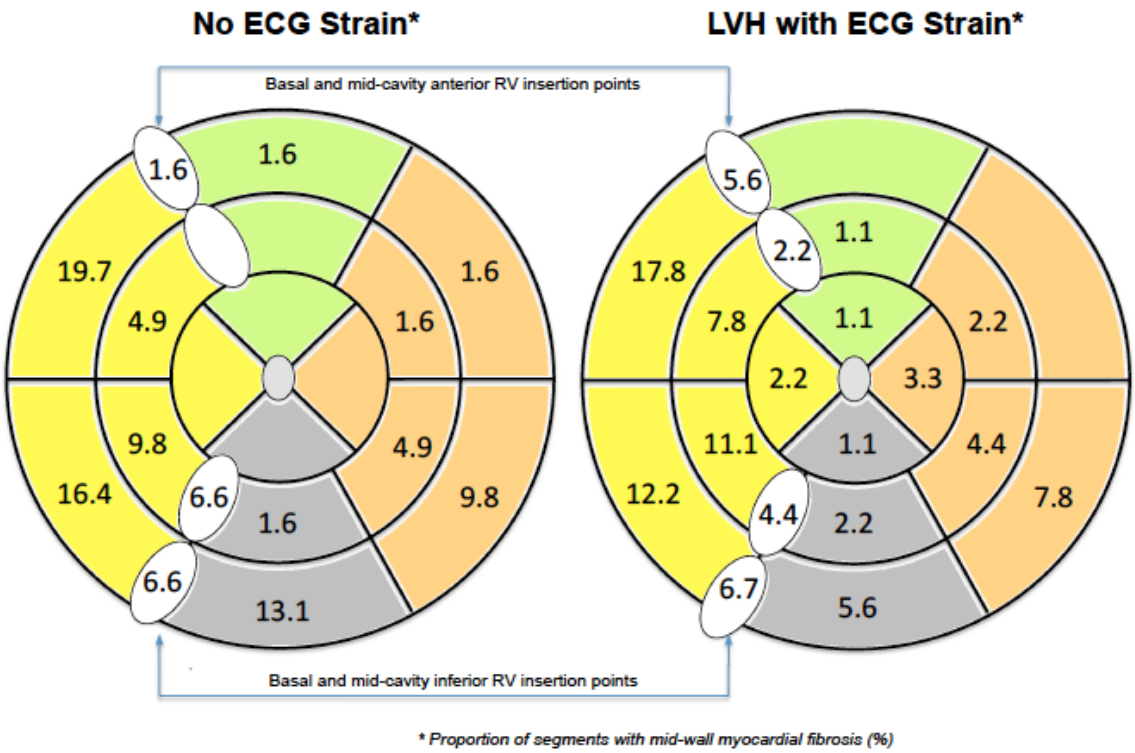
Aortic stenosis is a condition that affects not only the valve but also the myocardium. The transition from compensatory left ventricular hypertrophy to heart failure appears to be the key factor in determining the development of symptoms and adverse events. Assessment of this transition is currently limited, and interest surrounds the development of novel biomarkers of left ventricular decompensation. In a cohort of 102 patients with aortic stenosis, we have demonstrated that ECG left ventricular hypertrophy with strain is a highly specific marker of left ventricular decompensation and, in particular, of replacement myocardial fibrosis as assessed with cardiovascular magnetic resonance, a finding validated in an independent external cohort of 64 patients. Moreover, we have confirmed ECG strain as an independent predictor of cardiovascular mortality or aortic valve replacement in 140 asymptomatic patients recruited from the Scottish Aortic Stenosis and Lipid Lowering Trial Impact on Regression (SALTIRE) study. Our data indicate that a 12-lead ECG, which is readily available, cheap, and rapidly interpretable, can identify high-risk patients with aortic stenosis who potentially might benefit from early valve replacement.

SUPPLEMENTAL MATERIAL

DISTRIBUTION OF MID-WALL FIBROSIS

Mid-wall fibrosis was predominantly found in the basal and mid-cavity (92% and 100% of all segments with late gadolinium enhancement in patients with and without ECG strain, respectively). Whilst late gadolinium enhancement was observed more commonly in the septum, inferior and inferolateral than anterior segments, the distribution was not different between those with and without ECG-strain (P=0.78).

FIGURE S1

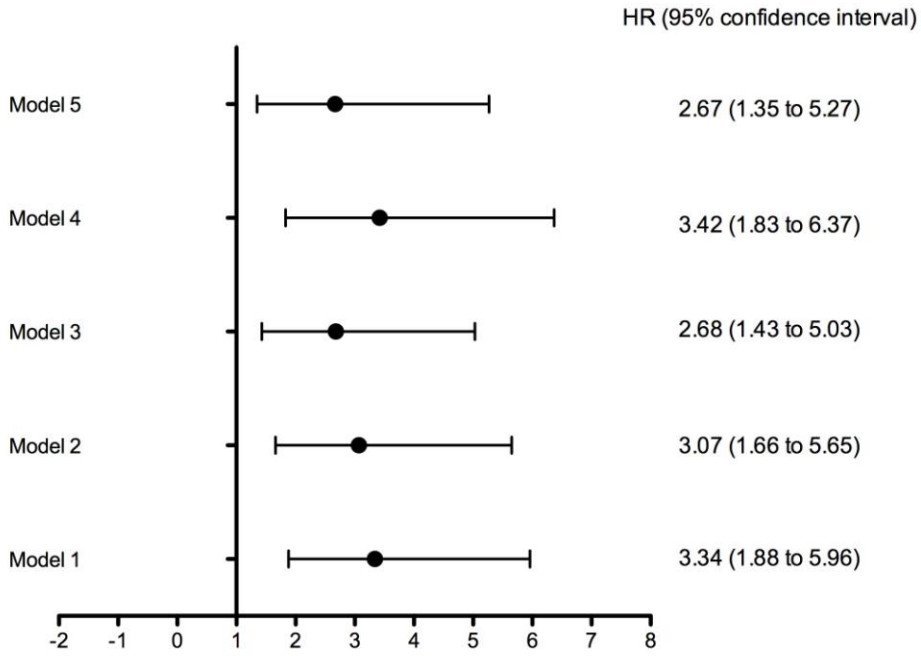


** Proportion of segments with mid-wall myocardial fibrosis (%)*

UNADJUSTED AND ADJUSTED COX MODELS PREDICTING TIME TO ADVERSE EVENTS

Further analyses were performed to determine the prognostic value of electrocardiographic strain in patients with aortic stenosis, adjusting for traditional markers of adverse outcomes (Figure S2).

FIGURE S2



Values presented are hazard ratios for the presence of LVH strain in predicting time to aortic valve replacement or cardiovascular death

Model 1 = unadjusted

Model 2 = adjusted for sex and age

Model 3 = adjusted as in Model 2 and aortic valve calcium score

Model 4 = adjusted as in Model 2, systolic ejection fraction and coronary artery calcium score

Model 5 = adjusted for aortic valve calcium score, mean pressure gradient, systolic ejection fraction and left ventricular mass index